LB 942

TriStar² S

Modular Monochromator Multimode Reader

Operating Manual

61456BA2

Rev. No.: 03, 04/2018

Not for use in in-vitro diagnostic (IVD) procedures.

The information in this guide is subject to change without notice.

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This instrument is not designed or intended for use with installations or equipment in hazardous environments. Servicing of the instrument must only be performed by Berthold Technologies Field Service Engineers or service staff authorized by Berthold Technologies.

Please contact our Service Center at service@berthold.com if you have any operational issues.

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1. Prefatory Comments

1.1 Explanation of LEDs and Beeps

LED	*Instrument status*
lights up **green**	Instrument OK and connection to PC OK
lights up **yellow**	Instrument OK, no connection to PC
flashes **yellow** + 1 short beep	New CAN is installed after power on of instrument
lights up **yellow** + 1 long beep	CAN correctly installed
lights up **red**	Shortly after power on of the instrument (during initialization)
flashes **red** + 2 short beeps	Error after power on of instrument / CAN module not correctly installed

1.2 Symbols on the instrument

The following hazard symbols and icons appear on the instrument:

Hazard symbol / Icon	*Description*
	**Warning - Before opening disconnect mains**
	***Warning - Risk of danger***
	***Warning - Optical radiation***
	***Warning - Hand Injury***
	***Warning – Biohazard material***
	***Warning - Hot surface***
CE	***This instrument bears the CE marks*** based on conformity to current EC legislation and stated on the declaration of conformity.
	***No domestic waste*** The electric product must not be disposed of in domestic waste.

1.3 Operating manual

This Operating Manual is structured as follows:

The manual covers all manipulations in a work flow order starting from installation via regular operation to maintenance.

In each section you are guided through the respective procedures step by step. The steps are consecutively numbered in each section. Explanations on the individual steps are added in small type font.

Explanations on the various types of operations are highlighted specifically.

For your convenience, illustrations are placed directly next to the respective text.

1.4 Typographical conventions

<**Add formula**>, <**OK**>, <**Close**>	**Buttons** are printed inside angular brackets in bold typeface
Menu **File**, **Open** dialog box	**Menu titles** and **dialog boxes** are printed in bold type
File | Open, **Options | Read**	**Menu items** are also printed in bold type; menu and submenu item are separated by a vertical line.

The following hazard symbols and icons can be found in the manual:

Hazard symbol / Icon	***Description***
	***Warning - Risk of danger***
	***Warning - Hot surface***
	***Warning – Biohazard material***
	***Warning - Corrosive***

2. Safety Instructions

2.1 Safety Instructions

Hot surface: Care while touching the cover or the lamp, it can be hot.

Caution! This symbol alerts the user to take special care on the very important issues of the manual. This operating manual includes information and warnings that have to be observed by the user in order to ensure safe operation of the instruments.

Please act always according to the following safety instructions, before as well as during operation of the system! Before set up and operation of the instrument it is necessary to read the instructions in this manual as neither safe operation of the instrument nor safety of the user are guaranteed otherwise. Failure to follow the instructions may invalidate the warranty

The instruments have been manufactured in accordance with the safety requirements for electrical measuring systems. If the law lays down regulations on the installation and/or operation of sample measuring systems, then it is the operator's responsibility to adhere to them.

The manufacturer has done everything possible to guarantee that the equipment functions safely, both electrically and mechanically. The user has to make sure that the instrument will be set up and installed properly to guarantee safe operation.

The instruments are tested by the manufacturer and are supplied in a condition that allows safe and reliable operation.

- ❑ This equipment must be installed and used in accordance with the manufacturer´s recommendations. Installation must be performed by properly trained and authorized personnel
- ❑ The instrument may only be operated by personnel who have been trained on the use of the system. It is strongly recommended that all users read this manual prior to use.

- ❑ **Never put parts of your body or other devices into the instrument while the unit is in operation.**
- ❑ **Remove the transportation lock before switching on the instrument.**
- ❑ The instrument is designed for indoor use only.
- ❑ **BERTHOLD TECHNOLOGIES** assumes no liability for any damages, including those to third parties, caused by improper use or handling of the instruments.
- ❑ The user is responsible for connecting the instrument in accordance with the valid regulations for electrical instruments.
- ❑ Set the instrument up to ensure easy access to the mains switch
- ❑ The mains supply voltage range of 100 - 240 VAC ± 10%, 50 / 60 Hz, Class I must not exceed.
- ❑ The instruments are designed according to these standards:
  IEC / EN 610 10-1: 2001 (2ed),
  CAN / CSA C22.2 No 61010-1-04,
  UL 61010-1, 2nd Edition"

- To disconnect the unit from the power supply, the plug of the AC adaptor must be disconnected from the unit.
- Do not open any instrument doors as long as the instrument is in operation.

- The mains adapter is provided with a 3-pole grounded plug. If your wall outlet does not allow connection of a 3-pole plug, have a suitable wall outlet installed by qualified personnel or use an adapter for safe grounding. Please observe the safety specifications of the grounded plug. Set the instrument up to ensure easy access to the mains switch.
- Service and repair work may be carried out by qualified personnel only.
- The operator may only perform the maintenance work described in this user guide.
- There are no exchangeable electrical components in the instrument. In case of malfunction call authorized service personnel
- Use only parts described in this manual for servicing.
- Disconnect power supply before opening the instrument.
- Upon removal of the front and top parts of the housing no safety measures are in effect. Be aware of any moving parts. The interior of the instrument may reach temperatures that can cause burns. Some parts of the instrument may remain hot without visual indication for some time after the power has been turned off.
- The electronic unit of the detector generates high voltage. Do not touch it during operation!
- If you can see that the instrument has become unsafe to use, switch it off and disconnect it from power supply.
- If liquid gets inside the instruments, pull the power cord. Clean the unit or have it cleaned by an authorized service center.
- Protect yourself from electrostatic charge, as discharge could damage sensitive instrument parts, especially sensitive parts of the computer and electronics boards. This is especially true when working on device openings, e.g. filter openings.
- The ventilation slits must not be covered. A distance of at least 10 cm to neighboring units or walls must be maintained.
- **The units are not for use in in-vitro diagnostic (IVD) procedures. Use the instrument only for the designated application. Please refer to the intended use statement.**

- Observe all legal requirements for handling biological or chemical assay reagents, samples and waste.
- Prior any measurement/operation with reagent liquids an individual risk assessment has to be done by the user.
- Some assays, assay components or specimen may pose a biohazard, a risk of infection or other kinds of danger for the user. Always adhere to the safety precautions and recommendations for assay performance and temperature range, written in the assay's package insert. Wear appropriate protective equipment such as laboratory coats or chemical rubber cloves and act carefully to avoid chemical burn, contamination and potential infection.

- **The operator is responsible for the use of reagents. Follow strongly the liquid safety advices.**
- Use only reagents recommended by the kit manufacturer and in accordance with the kit manufacturer's instructions for the designated assay, for priming the injector lines or washing and cleaning.

- Do not use any flammable or explosive solutions or liquids whose mixture is flammable or explosive.
- Avoid spilling liquids on the outer surface, the plate carrier or other parts of the instrument. Wipe up all spills immediately and decontaminate the surfaces in cases of biohazard spilled liquids.
- Waste (when priming/washing the tubing) always has to be disposed properly: If a waste pump is installed, a bottle has to be connected. If no waste pump is present, a suitable prime plate has to be placed below the injectors during priming/washing.

- Liquid from priming/washing may be corrosive (see chapter “Cleaning Tubing”)
- Injector solutions may be pumped back only if the appropriate reagent bottle is connected.
- Dispose chemical and biohazard waste carefully and according to local legislation. It is recommended to treat potential biohazard waste by autoclaving.
- The instrument should be shipped in its own case. For transport all transportation locks (e.g. for the plate carrier) have to be installed.
- For instrument cleaning, please refer to the respective sections in this manual.
- Reliable instrument function can be guaranteed only when original spare parts are used.

The tests and service work recommended by the manufacturer has to be performed to make sure that the operator remains safe and that the instrument continues to work correctly. Any service and maintenance work not described in this user guide has to be performed by authorized service personnel.

2.2 Consignes de Sécurité

Surface chaude: Attention en touchant le couvercle ou la lampe – danger de brûlures

Attention! Ce mode d'emploi contient des informations et avertissements qui doivent être suivis par l'utilisateur afin de garantir un fonctionnement en toute sécurité des instruments. Ce caractère indique des points importants qui sont essentiels à l'attention de l'utilisateur.

Il est impératif de respecter les consignes de sécurité suivantes, non seulement avant la mise en service mais aussi pendant le fonctionnement de l'appareil! Avant l'installation et mise en service de l'instrument tous les utilisateurs des appareils sont tenus de lire d'abord ces instructions de service. Le cas échéant, le fonctionnement correct de l'appareil et la sécurité de l'utilisateur ne peuvent être garantis. Ne pas suivre ces instructions d'utilisation entraine une annulation de la garantie

L´appareil a été fabriqué conformément aux règles de sécurité en vigueur pour les appareils de mesure électroniques. Si des réglementations légales existent pour le montage et/ou l'utilisation d'instruments de mesure d'échantillons, il est de la responsabilité de l'installateur et de l'exploitant de les respecter.

Le constructeur a fait le nécessaire pour assurer le fonctionnement sûr des appareils (du point de vue électrique, électronique et mécanique). L'utilisateur est tenu de veiller à ce que les appareils soient installés correctement afin d'éviter toute altération de leur utilisation sûre de garantir leur utilisation en toute sécurité.

Les appareils sont contrôlés à l'usine et livrés dans un état assurant sa sécurité de fonctionnement.

- Les appareils ne doivent être utilisés que par des personnes autorisées et leur utilisation est réservée au personnel compétent. Tous les utilisateurs des appareils sont tenus de lire d'abord ces instructions d'utilisation.
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- **Ne mettez jamais une partie de votre corps ou des objets dans l'appareil lorsque celui-ci est en fonctionnement.**
- **La fixation de transport doit être démontée avant la mise sous tension de l'appareil.**
- L'appareil est destiné uniquement pour une utilisation en intérieur.
- BERTHOLD TECHNOLOGIES décline toute responsabilité de dommages résultant d'une utilisation non conforme à l'emploi prévu, y compris des dommages causés à un tiers.
- Il est de la responsabilité de l'utilisateur d'installer l'équipement conformément à la réglementation électrique.
- Positionner l'appareil de manières à ce que les interrupteurs soient accessibles.

- La plage de tension d'alimentation du secteur ne doit pas dépasser
  100 - 240 VAC ± 10%
  50 / 60 Hz
  Classe I
- Les instruments sont conçus selon ces normes:
  IEC / EN 610 10-1: 2001 (2ed)
  CAN / CSA C22.2 No 61010-1-04,
  UL 61010-1, 2ème édition
- Pour déconnecter l'appareil de l'alimentation électrique, la fiche de l'alimentation doit être retirée de l'appareil.
- N'ouvrez aucune porte de l'appareil tant qu'il est en fonctionnement.
- 
  L'instrument est fourni avec une fiche à 3 broches dont une prise de terre. C'est une règle de sécurité. Il est nécessaire que cette fiche puisse être branchée sur une prise reliée à la terre. Dans le cas contraire, veillez à faire appel à un électricien afin d'installer une telle prise. Il ne faut pas négliger cette consigne de sécurité.
- Les travaux d'entretien et de réparation devront être confiés exclusivement à des spécialistes dûment formés.
- Seuls les travaux d'entretien décrits dans le manuel peuvent être effectués par l'utilisateur.
- Il n'y a pas de composants électriques interchangeables dans l'appareil. En cas de dysfonctionnement, appelez un technicien agréé
- Seules les pièces spécifiées peuvent être utilisées.
- Débrancher l'alimentation avant d'ouvrir l'appareil.
- Si vous ouvrez l'appareil, votre sécurité et celle de l'appareil ne sont plus garanties (capôt et parois de l'appareil). Faites attention aux parties mobiles. L'intérieur de l'appareil et certaines pièces peuvent atteindre des températures pouvant provoquer des brûlures s'il y a contact. Même lorsque l'appareil est éteint, des parties peuvent rester chaudes alors qu'il n'y a pas d'indication visible de température élevée.
- L'unité électronique du détecteur génère une tension élevée. Ne pas la toucher pendant le fonctionnement!
- Si vous vous apercevez que le fonctionnement de l'appareil n'est plus sûr, il faut alors l'arrêter et le débrancher de la prise secteur.
- Si du liquide a pénétré dans l'appareil il faut immédiatement le dé-brancher. Il faut ensuite le nettoyer ou bien le faire nettoyer par une agence de service après-vente autorisée.
- Protégez vous des charges électrostatiques afin d'éviter de provoquer des décharges qui pourraient endommager des parties sensibles de l'appareil telles que les cartes électroniques ou PC. Ceci concerne en particulier lors d'ouvertures de l'appareils, notamment lors de la manutention des barrettes de filtres
- **L'appareil n'est pas prevu pour l'utilisation diagnostique in vitro et ne peut être utilisé que pour son usage initialement prévu.**
- Les fentes d'aération ne doivent pas être couvertes. Une distance de 10 cm au minimum doit être maintenue entre l'appareil et d'autres appareils ou parois.

- Respecter toutes la réglementation en vigueur concernant la manipula-tion des déchets biologiques, des réactifs et des prélèvements/échantillons.
- Avant la première mesure ou manipulation avec les réactifs, l'utilisateur doit effectuer une évaluation des risques
- Certains systèmes de tests, composants de tests ou échantillons peuvent potentiellement présenter un risque biologique, un risque d'infection ou un autre type de danger. Respectez toujours les consignes de sécurité et les recommandations relatives à la performance et à la température recommandée du test, inscrites sur la notice. Porter un équipement de protection approprié, comme des blouses de laboratoire et / ou des gants de protection contre les produits chimiques, et faire preuve de prudence pour éviter les brûlures chimiques, la contamination et les infections potentielles.
- **L'utilisateur assume la responsabilité exclusive de l'utilisation des réactifs.**
- Utilisez uniquement les réactifs recommandés par le fabricant du kit et conformément aux instructions du fabricant du kit pour le test choisi, pour l'amorçage des lignes d'injection ou le lavage et le nettoyage.

- Ne pas utiliser des liquides et / ou de mélange de liquides inflammables ou explosifs.
- Évitez les éclaboussures de liquides sur la surface extérieure, le porte-plaque ou d'autres parties de l'instrument. Essuyez immédiatement toutes les éclaboussures et décontaminez les surfaces en cas de d'éclaboussures de liquides présentant un danger biologique.
- Les déchets (lors de l'amorçage / lavage de la tubulure) doivent toujours être éliminés correctement: si une pompe à déchets est installée, une bouteille doit être connectée. Si aucune pompe à déchets n'est présente, une plaque vide et appropriée doit être placée au-dessous des injecteurs pendant l'amorçage / lavage

- Le liquide provenant du tuyau d'évacuation peut être corrosif (voir chapitre "Cleaning Tubing / lavage des tubulures")
- Les solutions à injecter peuvent être pompéessi le flacon de réactif approprié est connecté
- Éliminer les déchets chimiques et biologiques avec soin et conformément à la législation en vigueur. Il est recommandé de traiter les déchets potentiellement dangereux à l'autoclave.
- Le fonctionnement correct est garanti si et seulement si les pièces de rechange utilisées soient appropriées.
- Transporter l'appareil uniquement dans son emballage d'origine. Lors du transport, bloquer le support de plaques à l'aide de la vis d'arrêt.
- Pour le nettoyage de l'instrument veuillez vous référer au paragraphe correspondant dans ce mode d'emploi.

Afin d'assurer la sécurité de l'utilisateur et le bon fonctionnement des appareils, effectuer les travaux d'inspection et d'entretien recommandés par le fabricant. Toutes les mesures d'entretien et de réparation allant au-delà de celles spécifiées dans ce manuel sont réservées aux techniciens autorisés.

2.3 Sicherheitshinweise

Heiße Oberfläche: Vorsicht beim Berühren der Abdeckung bzw. der Lampe, sie können heiß sein.

Achtung! Dieses Zeichen weist den Benutzer auf wichtige Punkte hin, deren Beachtung unerlässlich ist. Die vorliegende Bedienungsanweisung enthält Informationen und Warnhinweise, die vom Benutzer befolgt werden müssen, um einen sicheren Betrieb der Geräte zu ermöglichen.

Handeln Sie immer gemäß der vorliegenden Sicherheitshinweise, sowohl vor als auch während des Gerätebetriebs. Vor Inbetriebnahme des Gerätes ist es zwingend erforderlich, die Bedienungsanleitung zu lesen, da ansonsten die Sicherheit des Gerätes und des Benutzers nicht gewährleistet werden. Wenn Sie den Angaben in der Bedienungsanleitung nicht folgen, kann die Garantie erlöschen.

Die Geräte wurden in Übereinstimmung mit den Sicherheitsanforderungen für elektrische Messgeräte hergestellt.

Bestehen für die Errichtung und/oder den Betrieb von Probenmessgeräten gesetzlich vorgeschriebene Regelungen, so liegt es in der Verantwortung des Errichters und Betreibers, diese einzuhalten.

Der Hersteller hat alles unternommen, um ein sicheres Arbeiten der Geräte (bezüglich Elektrik, Elektronik und Mechanik) zu gewährleisten. Der Benutzer muss dafür sorgen, dass die Geräte so aufgestellt und installiert werden, dass ihr sicherer Gebrauch nicht beeinträchtigt wird.

Die Geräte sind werksgeprüft und wurden in betriebssicherem Zustand ausgeliefert.

- ❑ Die Geräte dürfen nur in Übereinstimmung mit Herstellerempfehlungen installiert und benutzt werden. Die Inbetriebnahme darf nur von ordnungsgemäß trainierten und autorisierten Personen durchgeführt werden.
- ❑ Die Geräte dürfen nur von dafür geschultem Personal betrieben werden. Es wird allen Anwendern dringend empfohlen, diese Bedienungsanleitung vor Benutzung zu lesen.

- ❑ **Während des Gerätebetriebs dürfen niemals Körperteile oder andere Geräte in das Instrument eingebracht werden.**
- ❑ **Die Transportsicherung muss entfernt werden bevor das Gerät eingeschaltet wird.**
- ❑ Die Geräte dürfen nur innerhalb von geschlossenen Räumen betrieben werden.
- ❑ Berthold Technologies übernimmt keinerlei Gewährleistung für Schäden, auch gegenüber Dritten, die durch unsachgemäße Handhabung der Geräte hervorgerufen werden.
- ❑ Es liegt im Verantwortungsbereich des Anwenders, dass die Geräte nach den lokalen elektrischen Vorschriften installiert werden.
- ❑ Stellen Sie das Gerät so auf, dass Sie es leicht ein- und ausschalten können.
- ❑ Die Netz-Stromversorgung darf den Spannungsbereich von 100 - 240 VAC ± 10%, 50 / 60 Hz, Class I nicht überschreiten.

- Die Geräte wurden entsprechend dieser Vorschriften entwickelt:
  IEC / EN 610 10-1: 2001 (2ed),
  CAN / CSA C22.2 No 61010-1-04,
  UL 61010-1, 2nd Edition
- Um das Gerät von der Stromversorgung zu trennen, muss der Stecker des Netzteils am Gerät abgezogen werden.
- Öffnen Sie das Gerät nicht solange es in Betrieb ist.
- Die Netzadapter sind mit einem 3-poligen Netzkabel ausgestattet. Dies ist eine Sicherheitsausstattung. Wenn die Steckdose keinen 3-poligen Anschluss unterstützt, muss ein Fachelektriker eine passende 3-polige Steckdose installieren oder einen passenden Adapter zur Erdung des Anschlusses bereitstellen. Zerstören Sie niemals die Sicherheitsvorkehrungen des geerdeten Anschlusses.
- Alle gelieferten Geräte und Zusatzgeräte sind geerdet ans Netz anzuschließen: Schutzkontaktstecker verwenden!
- Service- und Reparaturarbeiten dürfen nur von Fachleuten ausgeführt werden.
- Es dürfen nur die in diesem Handbuch beschriebenen Wartungsarbeiten vom Anwender ausgeführt werden.
- Es gibt im Gerät keine austauschbaren elektrischen Komponenten. Rufen Sie im Fehlerfall autorisiertes Servicepersonal.
- Bei Wartungsarbeiten dürfen nur die in diesem Handbuch angegebenen Teile verwendet werden.
- Vor dem Öffnen des Gerätes ist die Stromzufuhr zu unterbrechen.
- Wenn das Gerät geöffnet ist sind Sicherheitsmaßnahmen nicht mehr in Betrieb. Auf bewegliche Komponenten achten! Das Innere der Geräte kann Temperaturen erreichen, die Verbrennungen verursachen können. Einige Teile können heiß bleiben ohne sichtbare Zeichen, auch nachdem das Gerät abgeschaltet worden ist.
- Die Elektronik des Detektors erzeugt Hochspannung. Sie darf während des Betriebs nicht berührt werden.
- Bei Beeinträchtigung der Betriebssicherheit sind die Geräte abzuschalten und vom Netz zu trennen.
- Ist Flüssigkeit in das Innere des Gerätes gelangt, Netzstecker ziehen. Reinigen Sie das Gerät oder lassen Sie es durch eine autorisierte Servicestelle reinigen.
- Elektrostatische Aufladungen (z.B. durch Teppichböden) müssen beim Öffnen des Gerätes verhindert werden, da Entladungen am Gerät zur Beschädigung empfindlicher elektronischer Teile, besonders am Computer oder den Elektronik-Boards, führen können. Dies gilt besonders bei Arbeiten an Geräteöffnungen, z.B. Filteröffnungen.
- **Die Geräte sind nicht für den Einsatz in der In Vitro Diagnostik bestimmt und dürfen nur für den vorgesehenen Zweck eingesetzt werden.** Lesen Sie hierzu die Angaben zum bestimmungsgemäßen Gebrauch.
- Die Öffnungen des Ventilators dürfen nicht abgedeckt werden. Der Abstand zum Nachbargerät oder zur Wand muss mindestens 10cm betragen.

- Beachten Sie alle gesetzlichen Vorschriften für den Umgang mit biologischen oder chemischen Test-Reagenzien, Proben und Abfall.
- Vor der Messung/Benutzung von Reagenzien muss der Anwender eine individuelle Risikoanalyse durchführen.
- Einige Testsysteme, Testkomponenten oder Proben können potentiell eine biologische Gefährdung, ein Infektionsrisiko oder eine andere Art von Gefahr darstellen. Halten Sie immer die Sicherheitsmaßnahmen und die Empfehlungen für die Testdurchführung und den Temperaturbereich ein, wie sie in der Beilage des Testsystems angegeben sind. Tragen Sie angemessene Schutzausrüstung, wie Laborkittel oder Chemikalien- Schutzhandschuhe und arbeiten Sie vorsichtig, um chemische Verätzung, Kontamination und potentielle Infektion zu vermeiden.
- **Die Anwendung der Reagenzien liegt im alleinigen Verantwortungsbereich des Benutzers.** Befolgen Sie alle Sicherheitsanweisungen für Flüssigkeiten.
- Es dürfen nur vom Testhersteller empfohlene Reagenzien in Übereinstimmung mit den Angaben des Testherstellers für den ausgewählten Test, das Füllen der Injektorschläuche oder Waschen und Reinigen, verwendet werden.

- Verwenden Sie keine entflammbaren oder explosiven Lösungen, oder Flüssigkeiten deren Mischungen entflammbar oder explosiv sind.
- Vermeiden Sie das Spritzen von Flüssigkeiten auf die äußeren Oberflächen, den Plattenträger oder andere Teile des Instruments. Wischen Sie alle Spritzer sofort weg und dekontaminieren Sie die Oberflächen im Fall von verspritzten biogefährdenden Flüssigkeiten.
- Flüssigabfall vom Füllen oder Reinigen der Schläuche muss immer ordentlich entsorgt werden. Wenn eine Abfallpumpe installiert ist, muss eine Flasche angeschlossen werden. Falls keine Abfallpumpe vorhanden ist, muss ein passendes Auffanggefäß (prime plate) während des Füllens und Reinigens unter den Injektoren platziert werden.

- Flüssigkeiten, die aus dem Abfallschlauch kommen, können ätzend sein (siehe Abschnitt Cleaning tubing)
- Flüssigkeiten aus den Injektoren dürfen nur zurückgepumpt werden, wenn die entsprechende Reagenzienflasche angeschlossen ist.
- Entsorgen Sie chemischen und biogefährdenden Abfall vorsichtig und entsprechend der lokalen Gesetzgebung. Es wird empfohlen, potentiell biogefährdenden Abfall zu autoklavieren.
- Ordnungsgemäße Funktionalität kann nur bei Verwendung der Original-Ersatzteile garantiert werden.
- Das Gerät sollte nur in der eigenen Verpackung transportiert werden. Beim Transport ist darauf zu achten, dass alle Transportsicherungen (z.B. für den Plattenträger) installiert sind.
- Zum Reinigen des Gerätes bitte den entsprechenden Teil dieser Bedienungsanleitung beachten.

Für die Sicherheit des Benutzers und die Funktionsfähigkeit der Geräte sind die vom Hersteller empfohlenen Überprüfungen und Wartungsmaßnahmen durchzu-

führen. Alle über die Betriebsanleitung hinausgehenden Wartungs-und Instandhaltungsmaßnahmen dürfen nur von autorisierten Technikern ausgeführt werden.

3. Warranty and Technical Issues

3.1 Special spare parts

The following spare parts are safety parts: Use the original part from the manufacturer or direct agent only.

External power supply unit	**input** 100 - 240 VAC ± 10%; 50 / 60 Hz; Class I **output** 24 VDC, 9.2 A, max 221 W	GST220A24-R7B part no. 59048

3.2 Warranty statement

The instrument is sold in accordance with the general conditions of sale of Berthold Technologies GmbH & Co KG and its affiliates and representatives.
Berthold Technologies warrants this product to be free of defects in material and workmanship for a period of 12 months from the date of delivery, ex works Bad Wildbad.
Berthold Technologies or its authorized representative will repair or replace, at its option and free of charge, any product that under proper and normal use proves to be defective during the warranty period.

Berthold Technologies shall in no event be liable or responsible for any incidental or consequential damage, either direct or indirect.

The above warranty shall not apply if:

a) the product has not been operated in accordance with the operating manual
b) the product has not been regularly and correctly maintained
c) the product has not been repaired or modified by a Berthold Technologies authorized representative or user
d) parts other than original Berthold Technologies parts are used
e) the product and parts thereof have been altered without written authorization from Berthold Technologies GmbH & Co KG
e) the product has not been returned properly packed in the original Berthold Technologies packaging

This warranty does not apply to any third party product involved in the application.

Berthold Technologies reserves the right to refuse to accept the return of any product that has been used with radioactive or (micro)biological substances, or any other material that may be deemed hazardous to employees of Berthold Technologies. Such products have to be properly decontaminated and marked. Before returning products to Berthold Technologies ensure the devices are properly decontaminated and the form "**Confirmation on decontamination**" is properly filled in and will be accompanying the product. (See appendix for a blank form)

Before returning products to Berthold Technologies, a returns/repair number must be obtained and clearly identified on the packing and documents. Call Berthold Technologies to get this number. Retain the original packaging for use if the instrument needs to be returned to Berthold Technologies.

3.3 Customer service

Customer service will be provided in the first instance by the network of Berthold Technologies representatives. In the event of any problem experienced with your instrument, the first recourse should be **your local Berthold Technologies representative**. For further problems requiring hardware or software expertise, the Technical Support group at Berthold Technologies GmbH & Co KG will be available by phone, fax or email to deal with your queries. Here is their address, phone, fax and e-mail:

Berthold Technologies GmbH & Co KG
Technical Support
Calmbacher Str. 22
75323 Bad Wildbad
Germany
Phone: +49 7081 177 114
Fax: +49 7081 177 301
Email: service@berthold.com

At the end of this manual you will find a Customer Reply Form (Appendix section). If a problem arises with the instrument which you are not able to resolve, please fill in this form. This form should then be transmitted to your Berthold Technologies representative or to Technical Support at Berthold Technologies, where it will receive early attention.

Please also make sure that you have the relevant information available before contacting Berthold Technologies. Helpful information would include:

- serial numbers, part number, revision:
  see production label on instrument
- software and firmware versions
- monitor and log files (refer to the respective service manuals)

4. Introduction

4.1 Intended Use

The TriStar² is a modular multi-technology microplate reader for different types of fluorescent, luminescent and absorbance research applications.

The units are not for use in in-vitro diagnostic (IVD) procedures.

These units are not designed for use in hazardous areas.

4.2 Description

The **TriStar² S** microplate reader is distinguished by its exceptionally high sensitivity allowing detection limits in scientifically relevant magnitudes with low reagent consumption.

Detector sensitivity and stability are the result of Berthold Technologies´ experience with thousands of photon counters. The patent pending dual mode photodetector combines the advantages of true photon counting for high sensitive luminescence measurements with quasi background-free operation of the triggered analogue mode for best fluorescence results.

True photon counting has the benefit that no user parameters need to be set, ensuring the same conditions are used for every measurement during the instrument´s entire life time. The fast photon counting circuitry provides a dynamic range in excess of six orders of magnitude, which complements the range of the latest assays. For fluorescence measurements a pulse **triggered analogue circuitry** is implemented in the detector electronics, offering quasi temperature independent and noise-free operation.

A proprietary design of the optical system achieves absolute minimization of cross-talk down to 10^{-6} (depending on the type of microplate). A double grating monochromator in 3D design (option) can be used instead of filters for wavelength selection with variable settings for the slit widths for adjustable spectral bandwidths.

The instrument can read solid plates as well as strip plates from 6 to 384 well formats with a height **not** exceeding 21 mm (respective adapter frames need to be applied).

4.3 Recommendations for proper handling

To obtain good and consistent results please **follow these recommendations** :

- Do not expose instrument to direct sunlight
- Set up instrument in dry rooms
- Open lid for loading filter/microplates or cleaning only to keep light and dust out
- Keep plate carrier free from dirt
- Remove spilled reagents immediately with damp cloth or optical grade tissue
- Very bright samples may cause saturation of the PMT (indicated by an “Overload” message); let the PMT recover for a few seconds

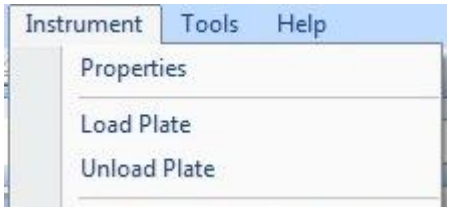
To avoid damages to mechanical, electrical and optical components **obey to these rules**:

- Load microplates correctly
- Do not use microplates or strip plates with heights exceeding 21 mm

- Do not fill the microplates above their specified maximum volume
- Do not shake completely filled microplates in the instrument
- Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system; take special care when ice in the trough starts to melt

4.3.1 Plate Tray

The instrument front panel includes the plate tray. It can be opened and closed under control of the **ICE** or ***Mikrowin*** software.



4.3.2 Filter Slides

Behind the big front flap the filter sliders are accessible. To replace or clean the filter you have to manually open the flap and eject the slides via software.

Proceed as follows

❒ Open the flap by hand; make sure the plate carrier is inside the instrument

❒ In the **Excitation Filter Slide** dialog box, click on the button <**Eject Slide**

❒ Clean or replace filter.

❒ Push in filter holder all the way into the slide.

❒ Click <**OK**> in the **Excitation Filter Slide** dialog box. The slide moves all the way into the instrument.

Do this with the emission filter in an analogous way

Cleaning filters

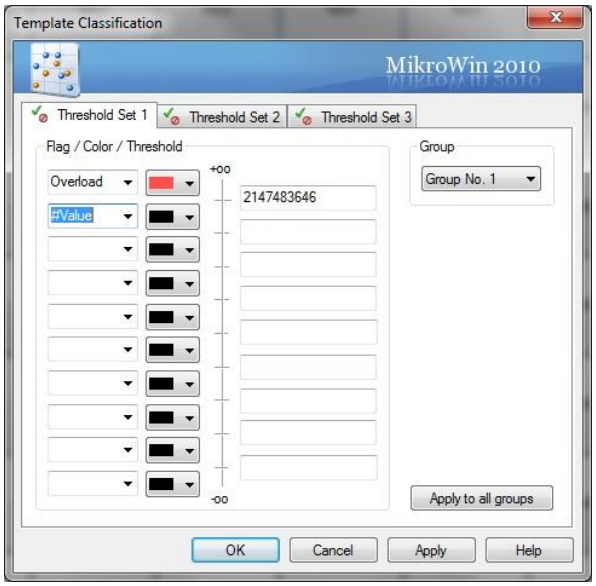
❒ Filters should be cleaned using a lint-free cloth or, better, a micro fibre cloth, as used for cleaning eye glasses.

4.3.3 Overload Detection

The detector has and **overload detection** function to prevent the PMT from damage by high levels of light.

Mikrowin supports this by displaying the expression ***Overload*** instead of a value. Instead of the expressions **MEA** or **LB,** in the calculation matrices one has to use the threshold function: **TRH (MEA)** or **TRH(LB1), TRH(LB2), …** respectively.

The threshold level itself and the expression to be displayed are set in the **Options | Threshold** dialogue (Type exactly: **2147483646** to guarantee maximal dynamic range without the risk of damage to the detector).



4.3.4 Injectors

The tubing from the solution bottles are connected to the injector ports using screw-type caps. The reagent trough and the reagent mounts provide means to position reagent vials safely.

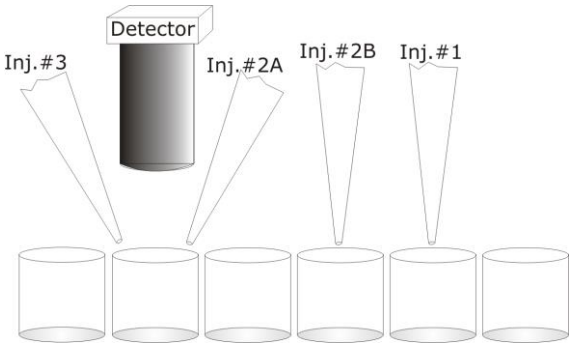
Injector parameters

The parameters for control of the injector are entered in the software:

- ❒ For measurement in the **Measurement Page** of the **Protocol Wizard (ICE)** or in **Settings (Mikrowin)**
- ❒ For washing and priming on the **Instrument** menu.

Injector Tip Location

The outlet-tips of the injectors are located right above to top level of the microplate The tips may be installed in different locations in horizontal orientation with respect to the measurement position:



5. Installation

Read this part completely prior to starting with the first steps and make sure that all pre-requisites are met as described below.

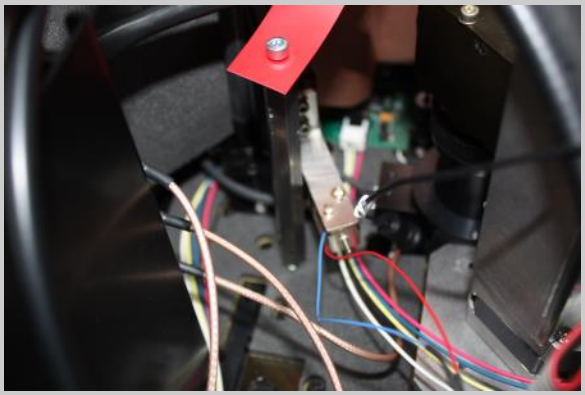
5.1 Unpacking and Set up

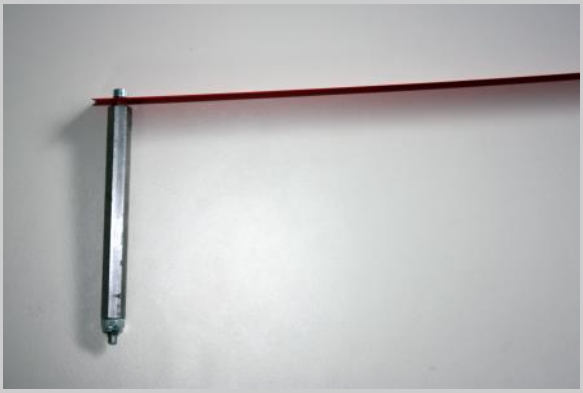
1. Unpack TriStar² S and accessories
2. The instrument is heavy and awkward to lift. It must be carried by 2 persons. Grab the device only from below (the device pedestals are raised therefor) and put TriStar² S onto an appropriate laboratory desk

3. Open the big front flap and **remove transportation safety device**

4. Open the top lid of the instrument my removing the four screws.

5. Remove the transportation lock indicated by the red tap. Make sure to unscrew the complete hexagonal rod attached to the horizontal support.





6. Remove external power supply from its box and connect to power cord. Connect the power cord to the respective socket of the instrument

7. Verify the mains switch is in **OFF** position

Mains switch Mains socket

8. Check if your mains supply is within the permissible range of the external power supply operating voltage (**100 – 240 VAC ± 10%; 50 / 60 Hz; Class I**)

   Connect instrument only if it is matching!

9. Put the jack of the external power supply into the wall outlet

10. For the consecutive software installation the instrument should remain **turned off**.

5.2 Software Installation

The instrument can be run with either ICE or Mikrowin software. Dependent on your software configuration follow either the instructions for ICE software or Mikrowin software installation respectively.

5.2.1 Installation of ICE operating software

Note: The software requires a computer with Microsoft Windows operating system (Windows 7, Windows 8, Windows 8.1, Windows 10). For installation local administrator level is recommended but not necessary.

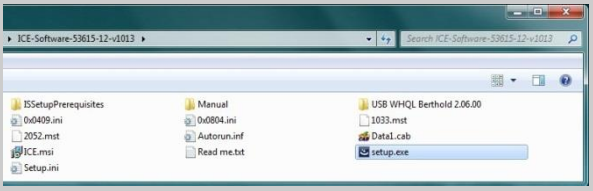
Note: As the software requires some additional resources for proper operation the set up wizard will check for the presence of these resources (**.NET Framework 2.0** and **Crystal Reports for .NET Framework**) on the computer. If the resources are found the installation of **I**nstrument **C**ontrol and **E**valuation (**ICE**) software is started.

In case these resources are not available on the computer the set up wizard will start with the installation of these resources.

1. Close all Windows applications before you start installing the software

2. **Insert software CD** into a CD or DVD drive
   The set up routine starts automatically

   In case the installation does not start automatically browse to the CD´s root directory and double click **Setup.exe**



3. Click **<Run>** when the Security Warning dialogue appears

   This or similar dialogues may appear during consecutive steps of the installation due to Windows security settings. Always confirm the messages to continue the installation.

4. Choose a **language**
5. Click **<OK>**

6. InstallShield will configure itself

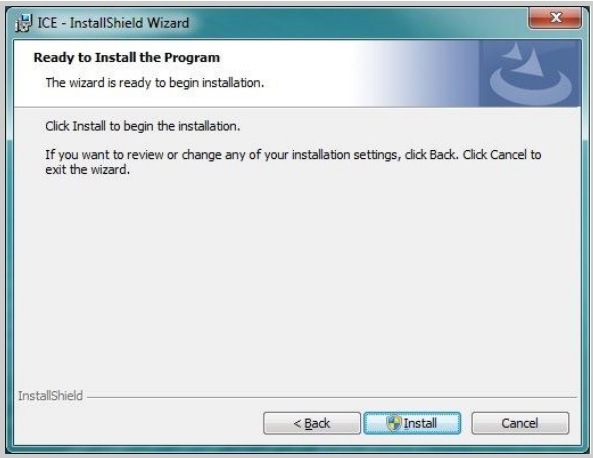
7. Click **<Next>** to start installation procedure

8. Choose an **installation directory**

   Due to support issues it is recommended to use the defaulted one

9. Click **<Next>**

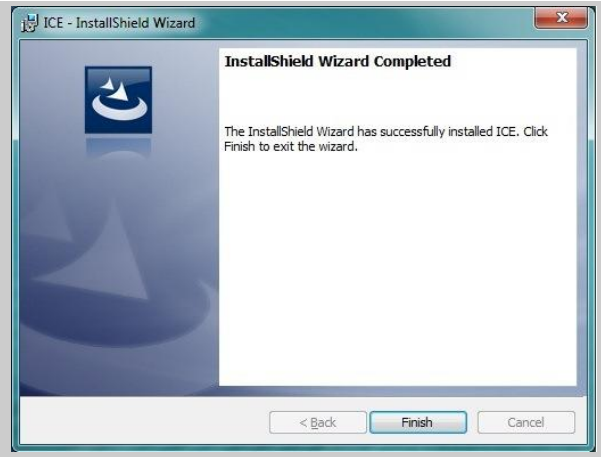
10. Click **<Install>**



11. The installation procedure will be executed automatically

Click **<Accept>** or **<Run>** should any warning messages appear

12. Click **<Finish>**



13. After a successful installation an **ICE icon** will be visible on the desktop

5.2.2 Installation of Mikrowin 2010 operating software

Note: The software requires a computer with Microsoft Windows operating system (Windows 7, Windows 8, Windows 8.1, Windows 10). For installation local administrator level is recommended but not mandatory.

Note: For the installation of MikroWin and driver software as well as for any updates and upgrades of the respective software the user has to have ***local Administrator rights*** for the computer.

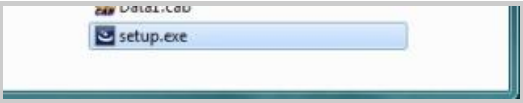
Note: ***Advanced*** versions are delivered with a hard lock (parallel or USB) for copy protection. The hard lock is matched with the installation CD. The hardlock needs to be attached during all operations with MikroWin. The ***Lite*** version needs to get activated with an activation code during or after installation.

Note: When a ***USB hard lock*** is used the installation has to be performed without the hard lock plugged in. The USB hard lock has to be put into the PC right after installation.

1. Close all Windows applications before you start installing the software

2. Insert software CD into a CD or DVD drive
   The set up routine starts automatically

   In case the installation does not start automatically browse to the CD´s root directory and double click **Setup.exe**

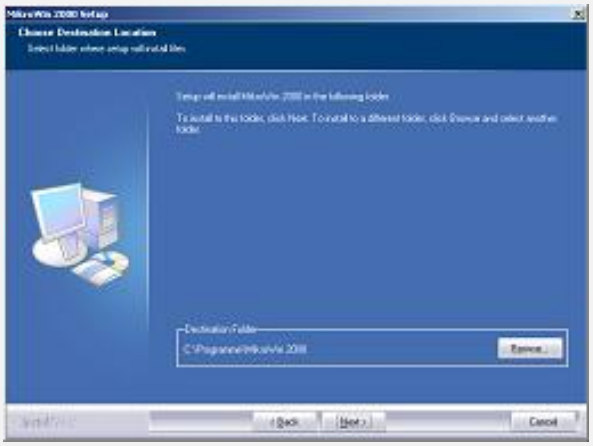


3. Select language and confirm with **<OK>**. The setup assistant is started
4. Enter name and company and click **<Next>**
5. Choose **destination location** (see screen shot to the right).

   The following path is defaulted
   C:\Program Files\Mikrotek\V50\Mikrowin2010

   For support reasons it is recommended to keep the default settings

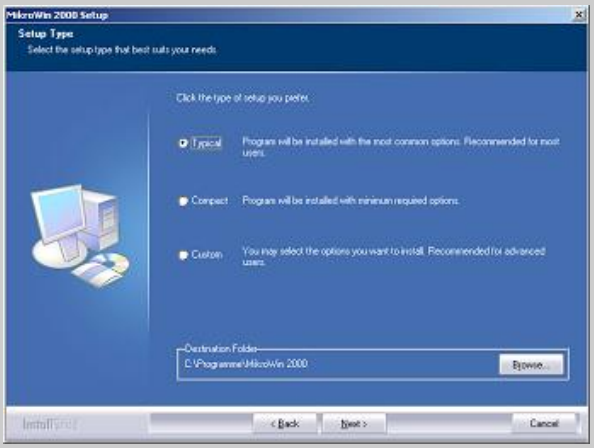
   If you wish to install the program to another folder, click **<Browse>** and select another folder
6. Click **<Next>**



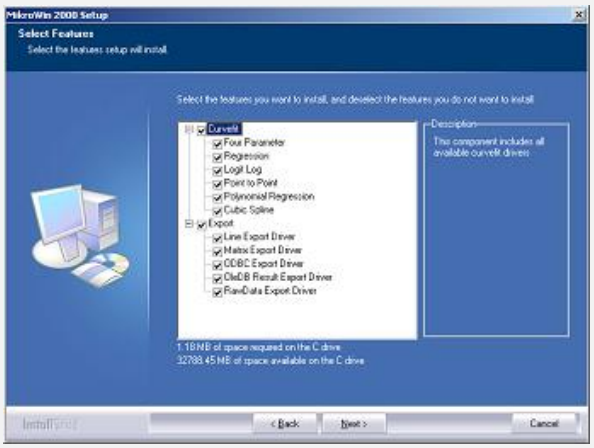
7. Select the **setup type**

   We recommend that you choose **Typical** for your first installation to ensure that all program components are installed.

   If you are familiar with the system, you may choose **Custom** to select the components you need for your application. You may especially **not** want to install the instrument drivers LB96V and Null Device.
8. Click **<Next>**



9. Select the desired components or deselect those components you don't want to install.
10. Click **<Next>**



11. Add program icon to the Mikrowin 2010 program folder.
12. Click **<Next>**.

    Installation is carried out and successful completion is indicated.

11. Click **<Finish>** to complete setup

12. Attach Mikrowin 2010 **USB hard lock** for ***Advanced*** versions

    or

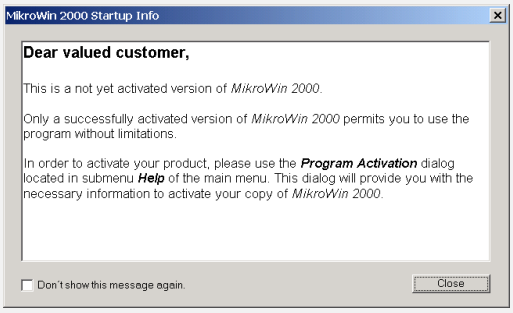
    Run the **Activation** procedure for ***Lite*** versions

5.2.3 Activation of MikroWin *Lite* Software

The Activation prodedure needs to be executed only when a new installation of Mikrowin 2010 has been performed.

1. This dialog will be displayed when starting a not yet activated **MikroWin 2010** ***Lite*** software without the instrument switched to on

   It is recommended to switch off and disconnect the **instrument** during software activation.

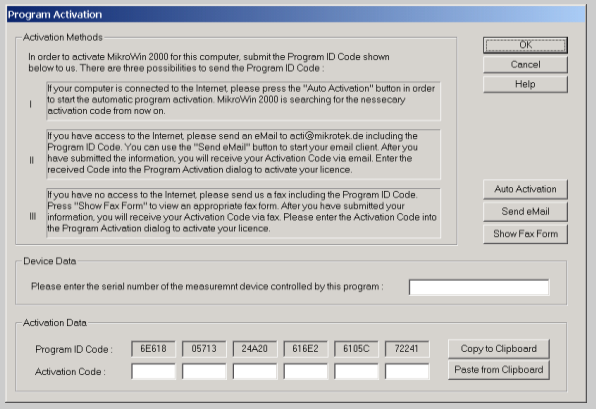


2. Go to **Help | Program Activation**

   There are 3 ways to acquire the activation code:
   I) online via internet (proceed with step **3**)
   II) via email (proceed with step **8**)
   III) via fax (proceed with step **16**)

   Activation via internet:

3. Enter serial number of instrument
4. Click <**Auto Activation**>
5. Click <**OK**> on the next screen displayed to con-



firm the activation process

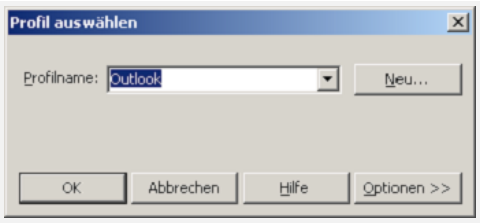
6. Code will be transferred online and will be automatically entered into the respective boxes

   Activation code will be returned within German office hours only

7. Once code is entered in respective fields click <**OK**>

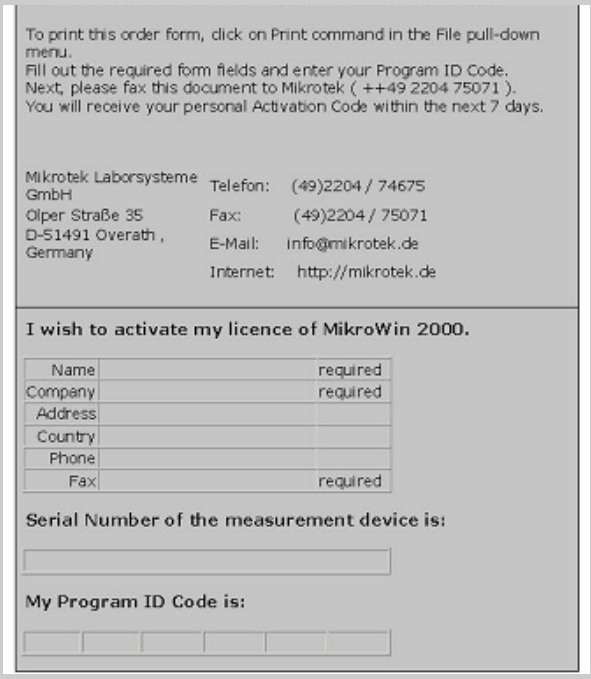
Activation via email:

8. Click <**Copy to Clipboard**>
9. Click <**Send eMail**>
10. Select suitable email profile
11. use "***MikroWin Program Activation***" as subject and provide these details of your system: ***Program ID Code***, ***Device Serial Number*** and ***Program Licence Code***
12. Email with respective activation code will be returned within 24 h
13. Copy code to clipboard.
14. Re-access the **Program activation** menu and click <**Paste from Clipboard**>
15. Click <**OK**>



Activation via fax:

16. Click <**Copy to Clipboard**>.
17. Click <**Show Fax Form**>.
18. Paste Program ID Code into respective fields and enter additional required information.


To print this order form, click on Print command in the File pull-down menu.
Fill out the required form fields and enter your Program ID Code.
Next, please fax this document to Mikrotek (++49 2204 75071).
You will receive your personal Activation Code within the next 7 days.

Mikrotek Laborsysteme GmbH
Olper Straße 35
D-51491 Overath ,
Germany

Telefon: (49)2204 / 74675
Fax: (49)2204 / 75071
E-Mail: info@mikrotek.de
Internet: http://mikrotek.de

I wish to activate my licence of MikroWin 2000.

Name		required
Company		required
Address		
Country		
Phone		
Fax		required

Serial Number of the measurement device is:

My Program ID Code is:

5.2.4 Installation of TriStar² S driver

In order to be able to communicate with the instrument via the USB port (executing operations and receiving data) the driver software needs to be installed and set up.

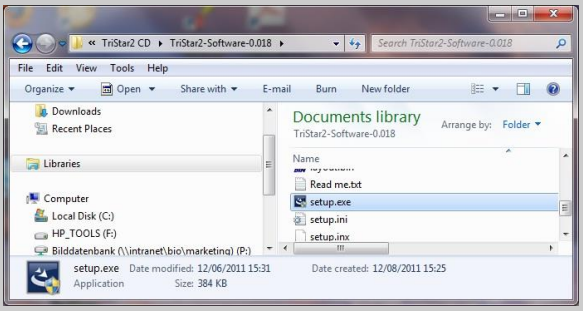
The instrument needs to be **switched off** during this process.

1. Close all Windows applications before you start installing the software
2. Make sure the instrument´s power switch is in **OFF** position

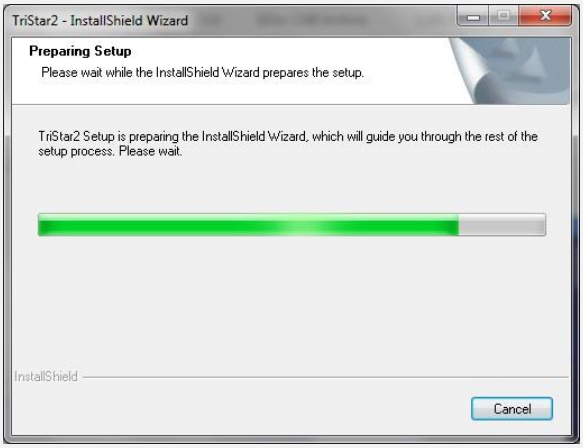
3. **Insert software CD** into a CD or DVD drive
   The set up routine starts automatically

   In case the installation does not start automatically browse to the CD´s root directory and double click **Setup.exe**

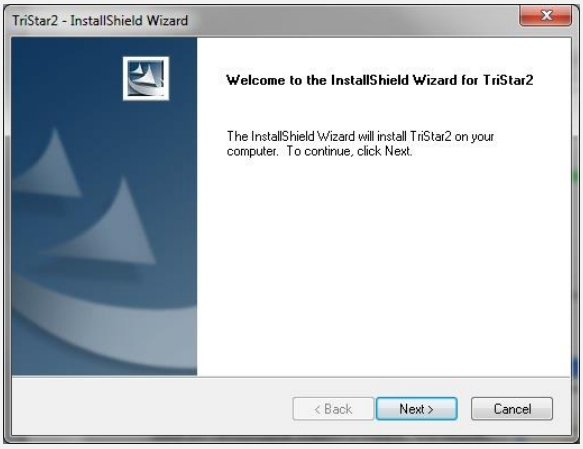
   Click **<Yes>** or **<Accept>** or **<Run>** should any warning message appear on your screen



4. Install Shield will prepare the installation

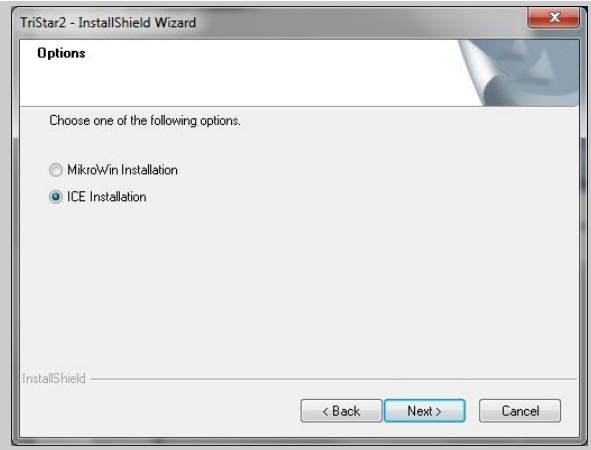


5. Click **<Next>**



6. Select the installation type for or **ICE** (or **MikroWin** depending on which kind of evaluation software you are using and have installed prior)

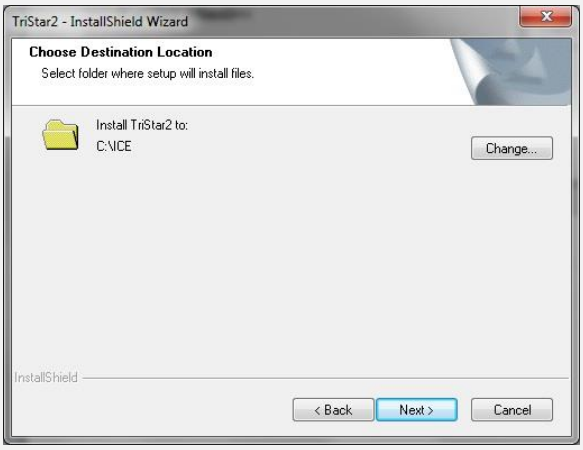
7. Click **<Next>**



8. Choose an **installation directory**

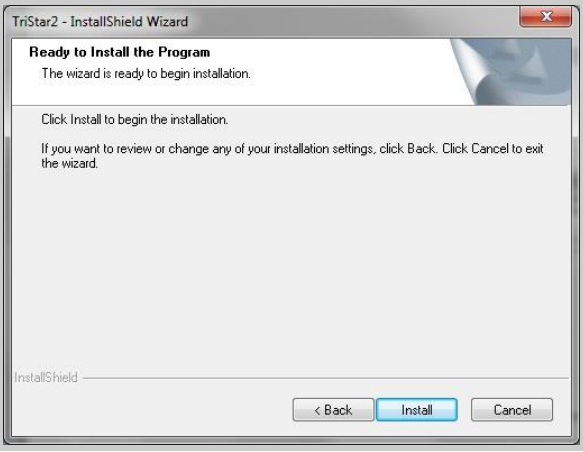
   Due to support issues it is recommended to use the defaulted one

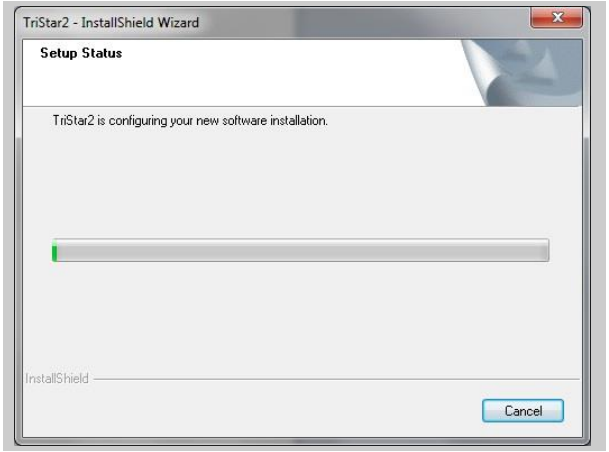
9. Click **<Next>**



10. Click **<Install>**

    Click **<Accept>** or **<Run>** should any warning messages appear

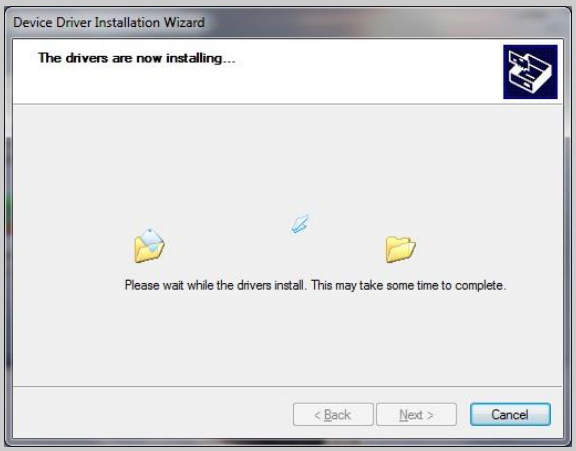




11. Click **<Next>**

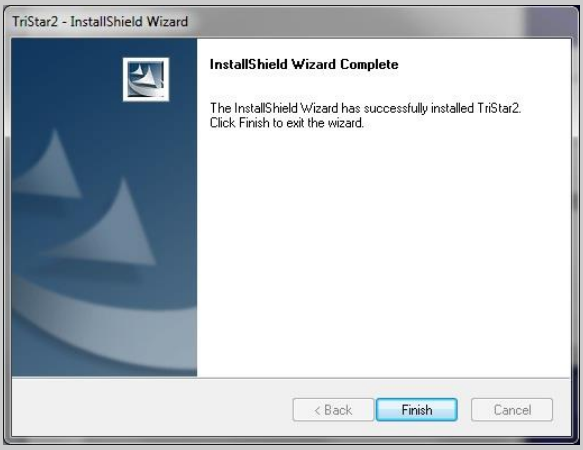
12. Wait for the installation procedure to be finished

Note: Certain Windows versions/configurations will show security dialog boxes such as “Do you want to allow the following program from an unknown publisher to make changes to this computer”. In this instance click **<Yes>**, **<OK>** or **<Run>**



13. Click **<Finish>**

14. Click **<Finish>**



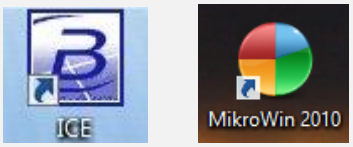
15. Connect the USB cable to a USB port of the computer

16. A message will be shown in the task bar during the USB driver installation

17. After a few minutes a message confirming the successful installation will be displayed in the task bar

18. Turn instrument on by putting mains switch into **ON** position

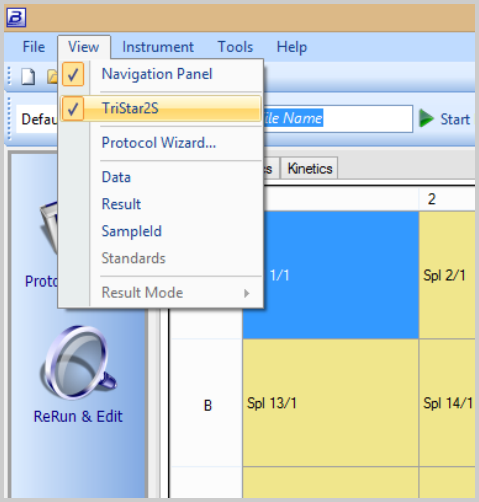
19. Open **ICE** software or **Mikrowin** software dependent on which kind of installation you have done prior



20. Select **TriStar² S** in **View** menu ***(ICE)***

    or

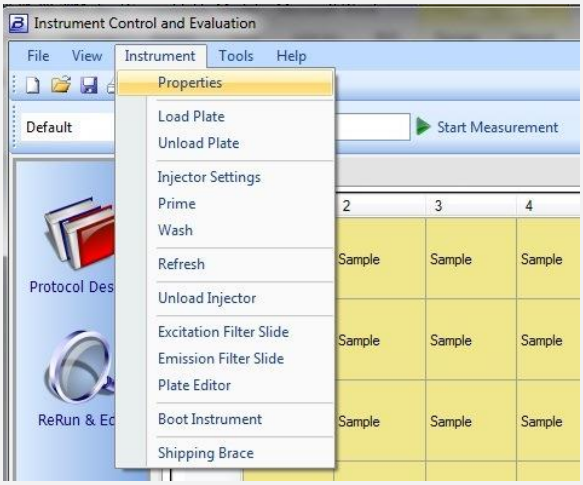
    Select the menu item **Installation | Driver *(Mikrowin)*** to open the Installation Driver dialog box with a separate tab for each driver type.



21. Go to **Instrument** menu and select **Properties *(ICE)***

    or

    Highlight **BertholdTech TriStar2S** and click on **<Driver Setup>** ***(Mikrowin)***



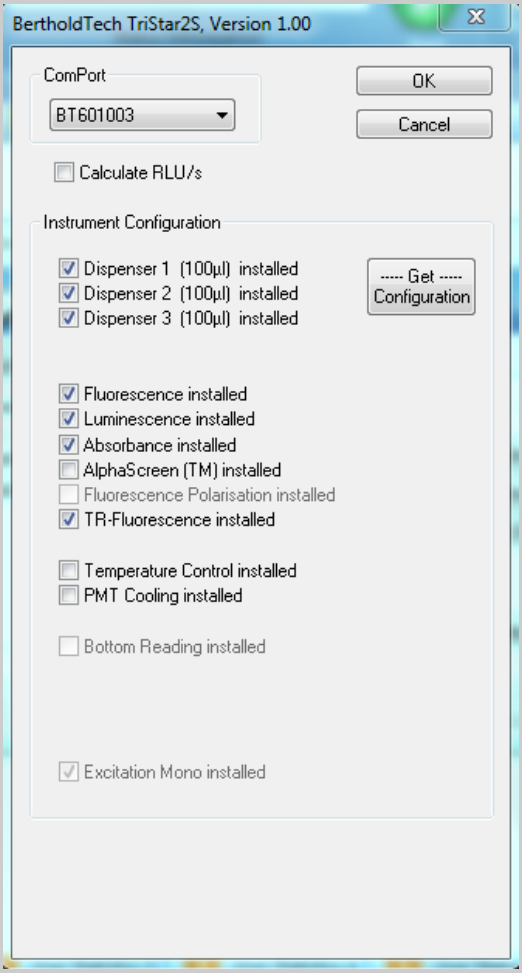
22. Select the entry starting with **BT60….** (e.g. BT601004) in the **ComPort** section

    The raw data are usually displayed as RLU representing the total amount of counts acquired during the reading time per well
    By checking **Calculate RLU/s** the total amount of counts will be divided by the respective reading time

23. Click <**Get Configuration**>
    the available injectors (with their volume) of the instrument will be automatically checked as well as Temperature Control and PMT Cooling when installed. Also, the measurement modules available in your instrument will be checked.

24. Click **<OK>**

25. ***Mikrowin only:*** Click **<OK>** to close the **Installation | Driver** dialogue



26. The instrument is now ready to use

5.3 Installing Filters

The instrument comes with an excitation and an emission filter slide, each of capable of holding up to 5 filters.

If the instrument is equipped with absorbance reading technology a 450 nm absorbance filter is included.

If the instrument is equipped with fluorescence reading technology a 485/14 nm excitation filter and a 535/25 nm emission filter are included.

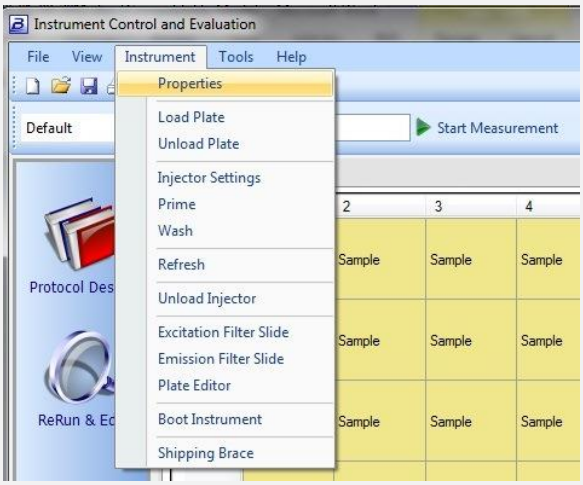
If the instrument is equipped with TRF a 350/60 nm excitation filter and a 615/8 nm emission filter are included.

If the instrument is equipped with TR-FRET/HTRF® a 320/40 nm excitation filter and two emission filters, 620/10 nm and 665/7 nm, are included.

In case additional filters are required they can be ordered individually and can easily be installed, both physically and in the software.

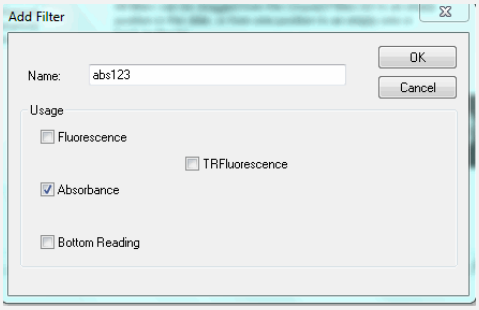
5.3.1 Excitation filters

1. Select **Excitation Filter Slide** in the **Instrument** menu

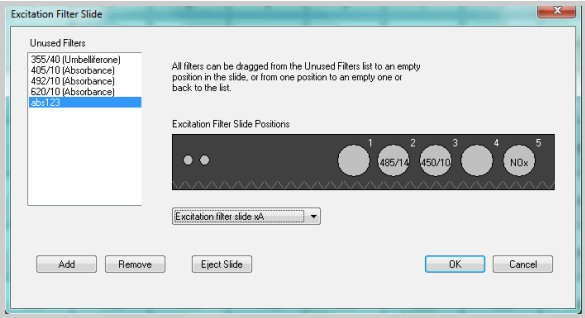


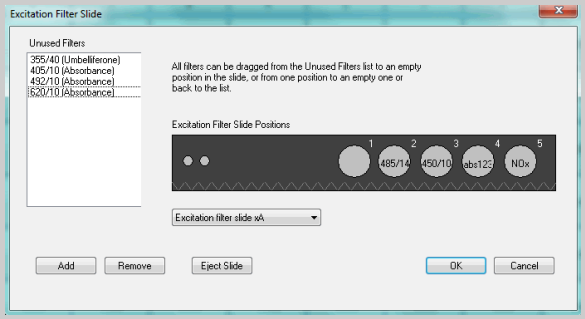
2. Click **<Add>**

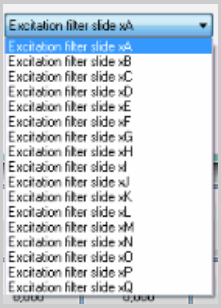
3. Define a (descriptive) **Name** for the new filter
4. Check the **Usage**
5. Click **<OK>**



6. Highlight the new filter in the Unused Filters list and drag it into an empty position of the filter slide.
7. Some filter slides are preconfigured for certain measurement technologies (xD = time resolved fluorescence (TRF) and TR-FRET, xE, xF, xG = fluorescence polarization (FP)).







8. Open the big flap at the front
9. Click **<Eject Slide>**
10. Remove excitation filter slide from the instrument

11. Mount the filter(s) into the position(s) defined in the software

    for excitation and absorbance, filters with diameters of 12.7 mm (½ inch), 15 mm or 25 mm (1 inch) can be used

    12.7 mm filters need to be mounted with a matching adapter (**ID 57194-005**) and a matching clamp ring (**ID 57195-005**)

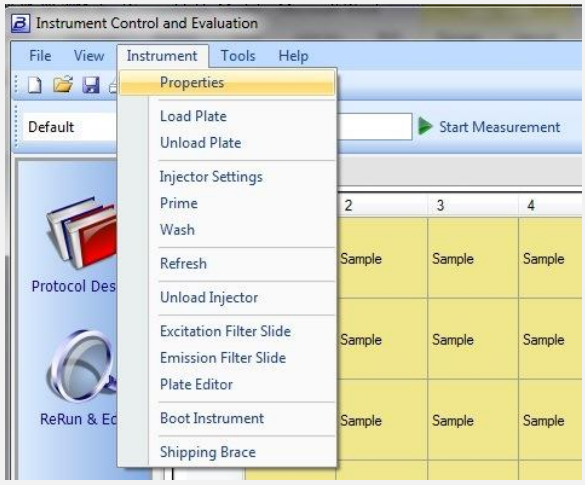
    15 mm filters need to mounted with a matching adapter (**ID 54666-005**) and a matching clamp ring (**ID 34767-005**) as well

excitation filters mounted

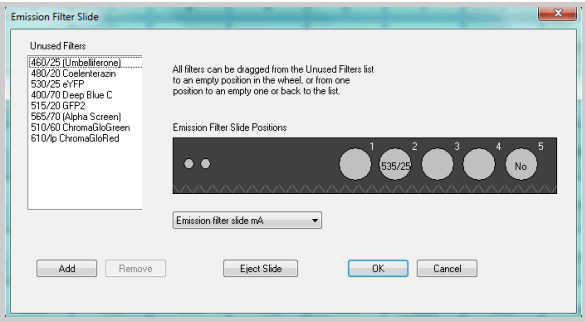
12. Insert the slide again until the front of the slide is aligned with the front of the instrument
13. Click **<OK>**
14. Close the front flap

5.3.2 Emission filters

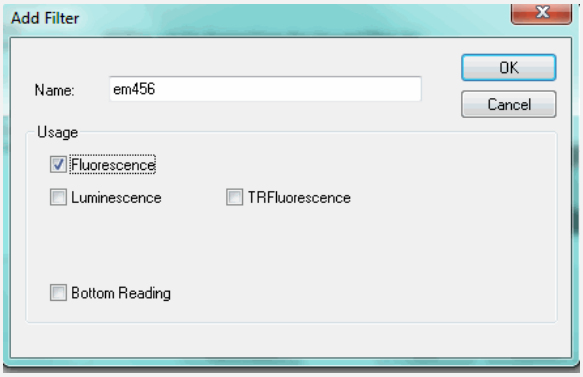
1. Select **Emission Filter Slide** in the **Instrument** menu



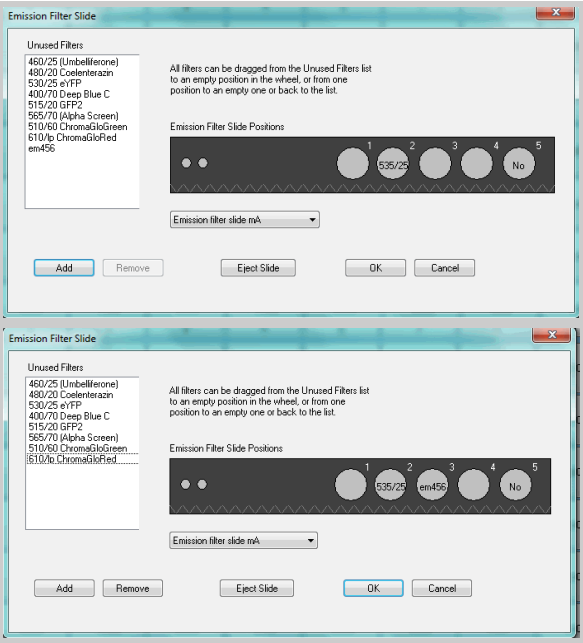
2. Click **<Add>**



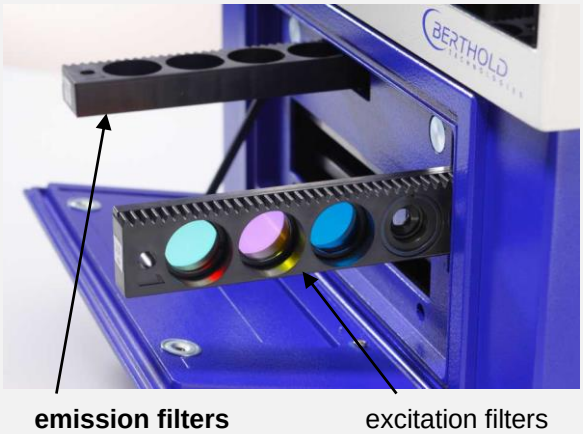
3. Define a (descriptive) **Name** for the new filter
4. Check the **Usage**
5. Click **<OK>**



6. Highlight the new filter in the Unused Filters list and drag it into an empty position of the filter slide



7. Open the big flap at the front
8. Click **<Eject Slide>**
9. Remove emission filter slide from the instrument



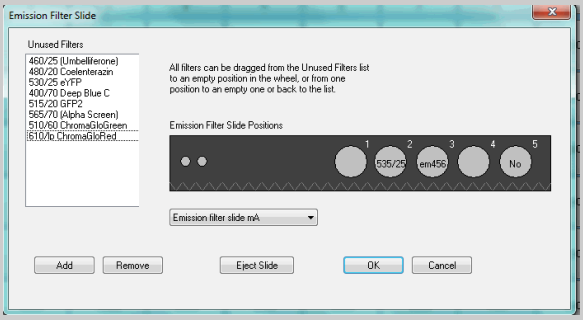
10. Mount the filter(s) into the position(s) defined in the software

    25 mm filters are recommended for emission as they are ideally matching the emission light path

    filters with diameters of 12.7 mm (½ inch) and 15 mm may be used but are not recommended as

Emission filters mounted

Position 5 is reserved for lumi-

sensitivity will be compromised 12.7 mm filters need to be mounted with a matching adapter (**ID 57194-005**) and a matching clamp ring (**ID 57195-005**) 15 mm filters need to mounted with a matching adapter (**ID 54666-005**) and a matching clamp ring (**ID 34767-005**) as well	nescence readings
11. Insert the slide again until the front of the slide is aligned with the front of the instrument 12. Click **<OK>** 13. Close the front flap	
14. Click **<OK**	
15. NOTE: Emission filter mH for bottom measurement position (see instructions in the description of the distinct measurement technologies for details) use special filter slides with apertures and integrated 45° degree mirrors to redirect the bottom emission to the detector. Filters may be mounted above these mirrors as described above.	

5.4 Bottom Reading Position

The TriStar²S LB 942 can measure microplates from the bottom reading position, exciting the sample and collecting the emission light from underneath the microplate. This measurement mode is available for selected readout technologies (see description of the respective technologies for details).

NOTE: To use the bottom reading position, make sure, an mH emission filter slider is installed and the red microplate frame is used.

6. Instrument Control and Evaluation Software

6.1 ICE Directories and Files

The directories for data and parameter files are defaulted as described below. Any accessible directory on the computer and the local network can be selected though when saving data and parameter files using the "**Save … File As…**" command.

Default directories

- Data files My Documents\ICE\DataTriStar2S
- Protocol files My Documents\ICE\ParaTriStar2S
- Priming files My Documents\ICE\ParaTriStar2S

In consequence each Windows user has own directories containing his data and protocol files. Hence, when users log on individually shared files may need to be copied to each user´s ParaTriStar2S directory, esp. the Default Customized Priming sequence **100default_01.wge**

File Names

There is no limitation in naming data and protocol files other than the Microsoft Windows conventions.

Data file names are to be defined prior to measurement start. Renaming is possible using the "**Save Data File As…**" command producing a copy of the data file with a new name.

Protocol file names are to be defined at the end of creating a protocol. Renaming is possible using the "**Save Protocol File As…**" command producing a copy of the protocol file with a new name.

File Types

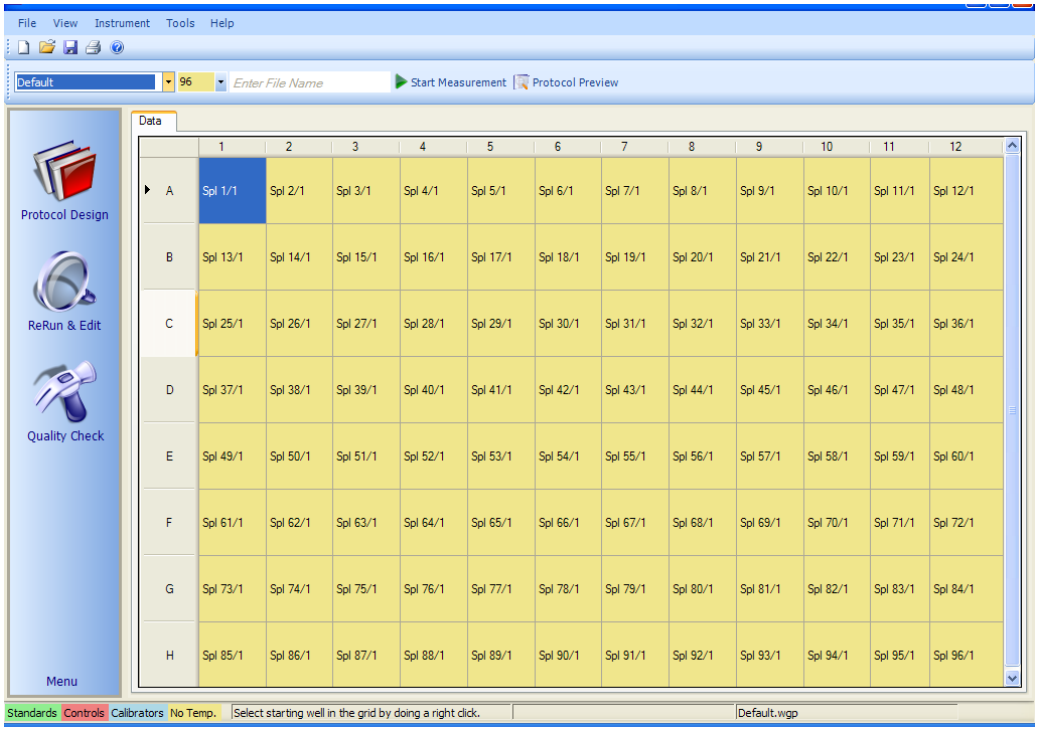
ICE works with 5 file types indicated by the respective file name extensions.

- Protocol files have the extension **.wgp**
- Data files have the extension **.wgd**
- Standard curve files have the extension **.wgs** (to be used as reference curves)
- Multiple Analyte profiles have the extension **.wgm**
- Customized prime sequences have the extension **.wge**

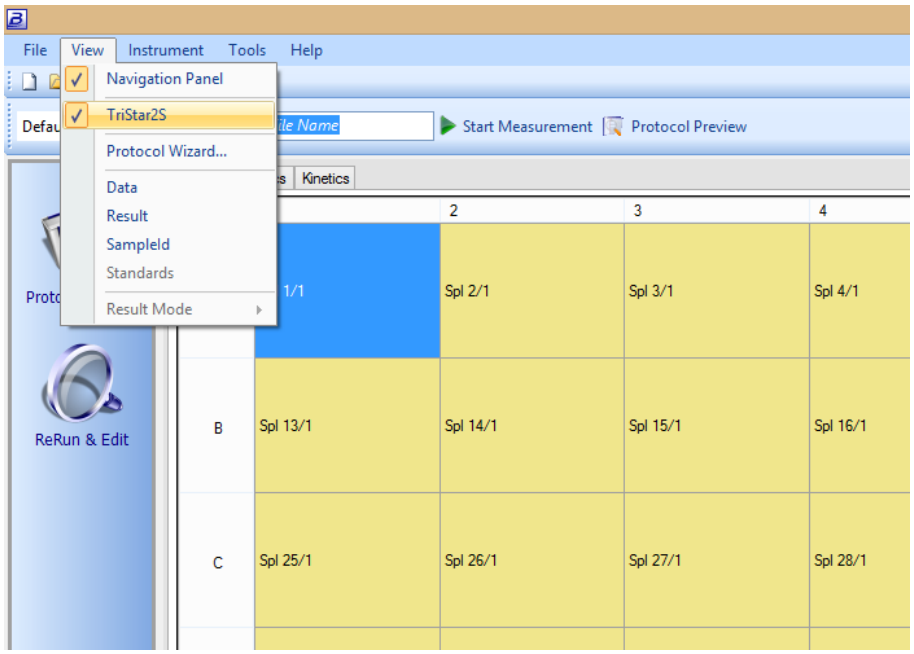
6.2 ICE User Interface

6.2.1 ICE default set up

The next figure shows the default start-up screen of ICE.

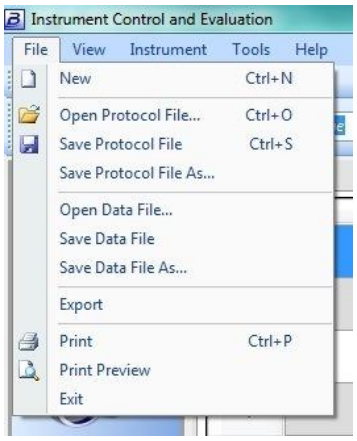


To return to the default layout after any changes may have been made check **Navigation Panel** and **TriStar² S** in the **View** menu.



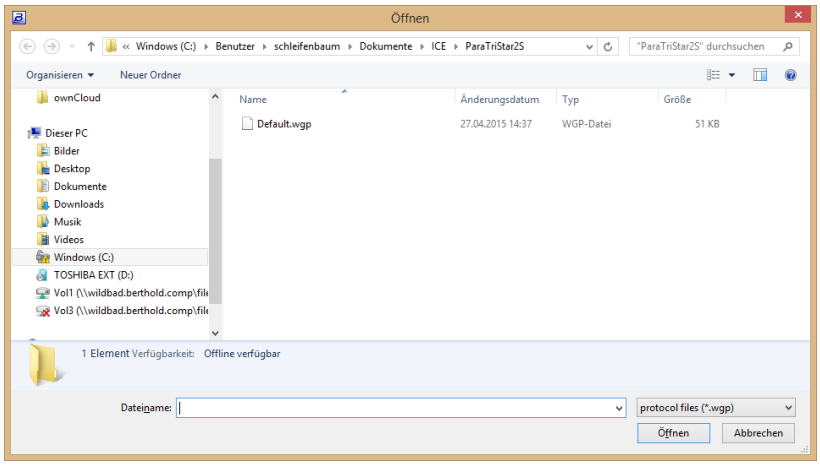
6.2.2 File menu

The **File** menu contains commands to open and save data and protocol files.



New clears data display to start a new measurement

Open Protocol File… opens an existing protocol



Save Protocol File saves loaded protocol file

Save Protocol File As… saves loaded parameter settings with a new name

Open Data File opens an existing measurement

Save Data File saves displayed data

Save Data File As… saves displayed data with a new name

Export exports the data set as EXCEL file according to the settings made in the protocol

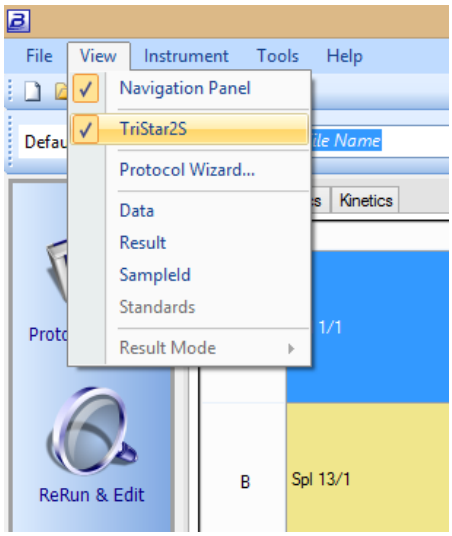
Print prints the selected data set shown on the screen

Print Preview displays a preview of the print-out

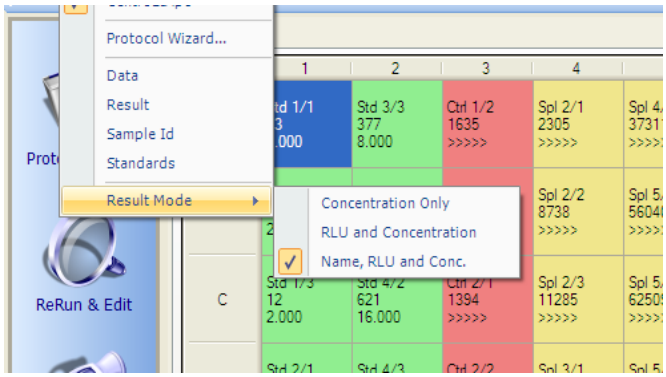
Exit closes ICE software

6.2.3 View menu

The **View** menu defines how the user interface and data are displayed.

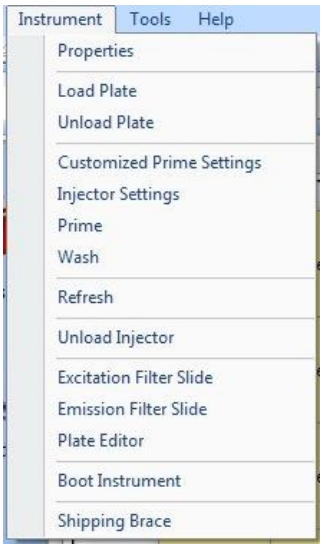


Navigation Panel	shows/hides navigation panel on the left
TriStar² S	adjusts user interface for TriStar² S
Protocol Wizard	starts wizard for protocol creation
Data	displays raw data (RLU or RLU/s)
Result	displays calculated data
Sample ID	displays sample IDs
Standards	displays standard concentrations
Result Mode	to select the content of the result display

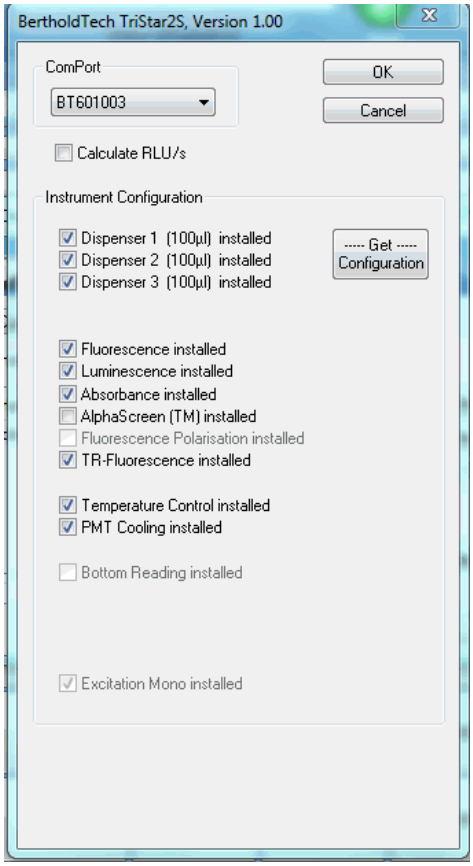


6.2.4 Instrument menu

In the **Instrument** menu basic instrument settings and communication may be accessed.



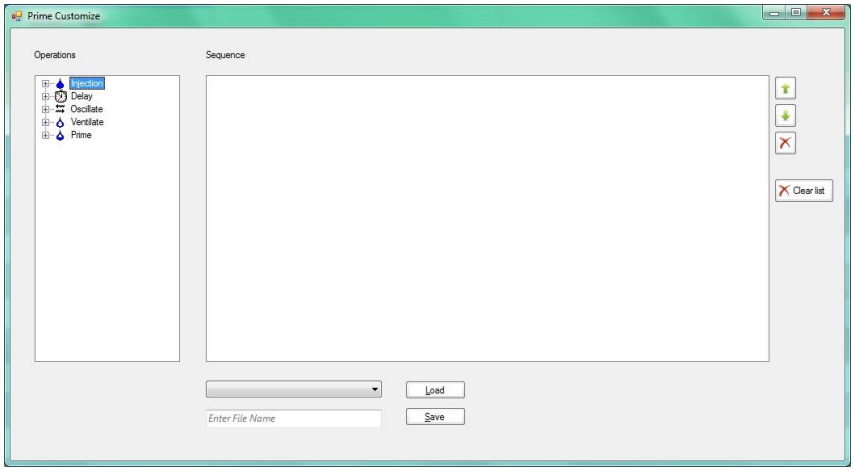
Properties instrument driver settings



Load Plate moves plate into the instrument

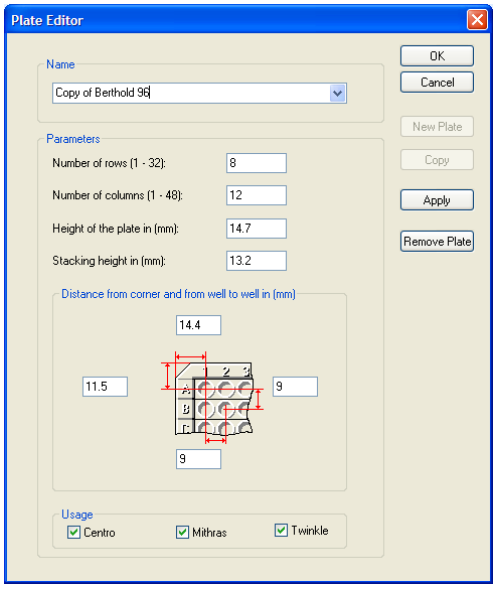
Unload Plate moves plate out of the instrument

Custom. Prime Settings dialogue for editing prime sequences



For the setting and options please read chapter "Priming Tubings"

Injector Settings	general settings for wash and prime sequences
Prime	starts the priming sequence (filling the lines)
Wash	starts the washing sequence (cleaning the lines)
Refresh	injects once to fill the tip (e.g. after longer periods of idleness)
Unload Injector	starts the unloading sequence (recovering reagents back into the reservoir)
Excitation Filter Slide	dialogue for definition and positioning of excitation filters
Emission Filter Slide	dialogue for definition and positioning of emission filters
Plate Editor	dialogue for definition of microplate dimensions



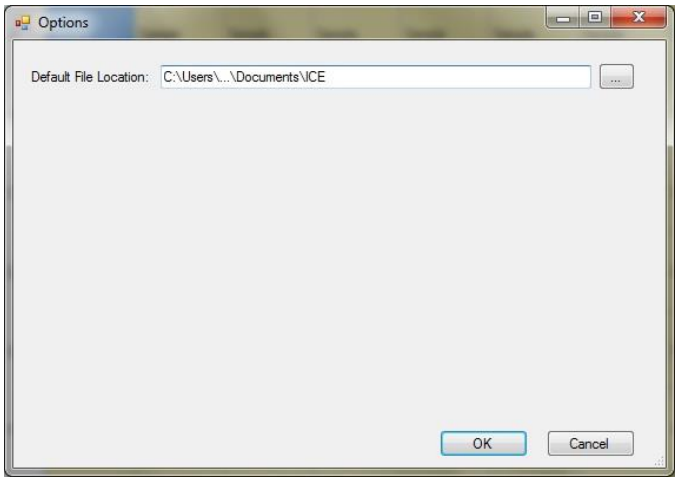
Note: only 6 to 384 well plates are supported in the TriStar² S. Petry dishes, Terasaki plates and filter membranes can be used, but have to be specified individually.

Note: only plate heights of up to 21 mm are supported in the TriStar² S

Boot Instrument	establishes communication and boots instrument
Shipping Brace	moves XY table to a position enabling the insertion of the transportation lock

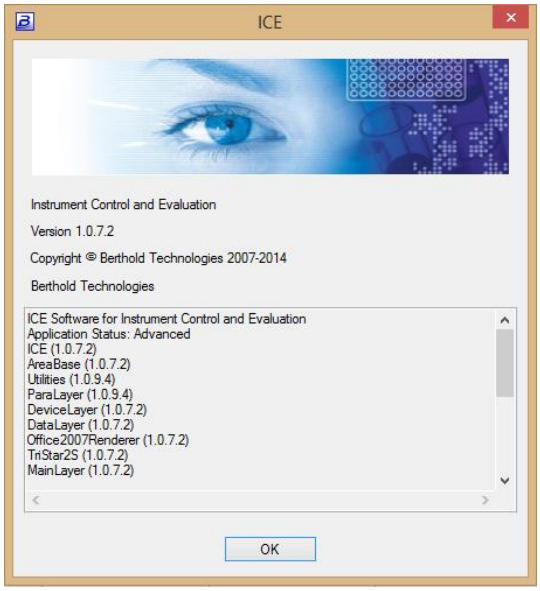
6.2.5 Tools menu

In the **Tools | Options** menu you can define the default root directory for the protocol (***ParaTriStar2S***) and data (***DataTriStar2S***) folders.



6.2.6 Help menu

The **Help** menu allows you to view basic software information.



7. Operation with ICE

Running measurements on the TriStar² S is straight forward. The procedure is the same for all types of assay types, e.g. Raw Data, Dual Label, Kinetic, Repeated, Scanning and Spectral Scanning. A measurement can be carried out immediately after a stored protocol is selected. At the end of each measurement the results are stored and may be printed or exported.

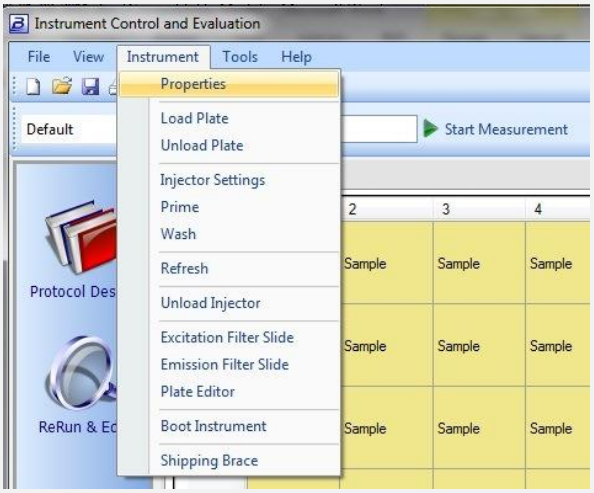
Result file names can be given without limitation. The extension is fixed, though. This is valid for measurement protocols as well.

7.1 Adding and Editing Microplate Dimensions

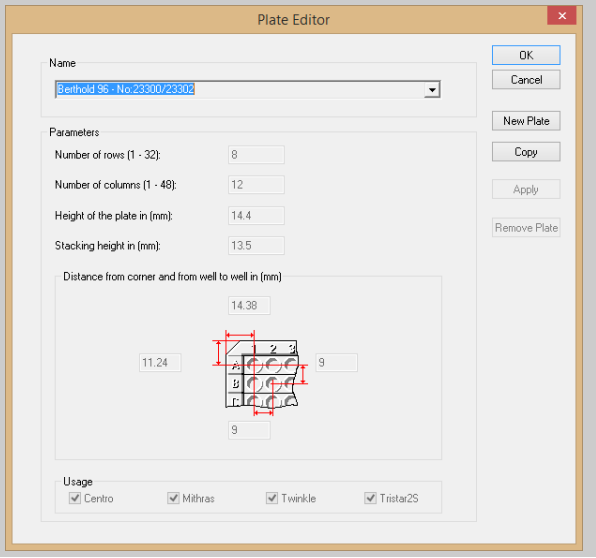
Microplates can differ in their dimensions dependent on brand and type. Please refer to the manufacturer´s most recent information for exact dimensions of the microplates.

Microplates must be defined in the plate editor prior to defining a measurement protocol.

1. Click **Plate Editor** in the **Instrument** menu



2. Click **<New Plate>** or select a plate with matching well format and click **<Copy>**



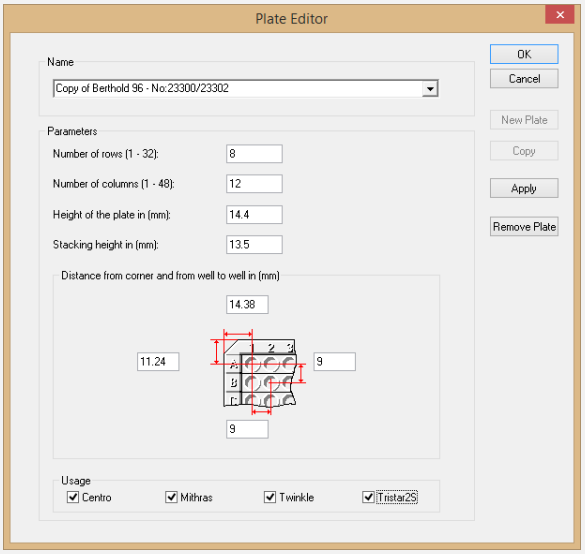
3. Assign a (descriptive) **Name**
4. Insert the **Number of rows**, e.g. ***8*** for a 96 well plate
5. Insert the Number of columns, e.g. ***12*** for a 96 well plate
6. Insert the total **Height** of the microplate

   most 96 and 384 well plates are between 14 and 15.5 mm
7. Insert the **Stacking height** of the microplate

   the stacking height is the resulting height (the visible part) when plates are put on top of each other (e.g. in a plate stacker)

   in case this information is not available from the plate manufacturer the stacking height can be derived by stacking 2 plates and measuring the total height; by subtracting the regular height of one of the plates the resulting value will be the stacking height
8. Insert the distance between the left outer edge of the plate and the center of well A1
9. Insert the distance between to upper outer edge of the plate and the center of well A1
10. Insert the distance between the well centers of consecutive rows (vertical well distance)
11. Insert the distance between the well centers of consecutive columns (horizontal well distance)
12. Check the usage ***TriStar2S***

    you may check additional instruments in case you have multiple instruments in operation
13. Click **<Apply>**
14. Click **<OK>**



15. The plate can now be used in the protocol files

7.2 Single Raw Data measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine ATP content, single reporter gene expression, activities of caspases, kinases and many other enzymes.

7.2.1 Defining a Single Endpoint protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

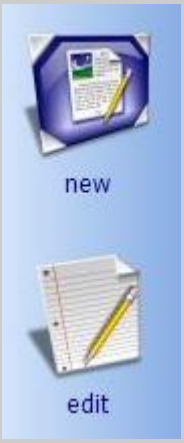
1. Click icon **Protocol Design** in the left-hand **Navigation** bar

   the navigation bar will appear in a new design

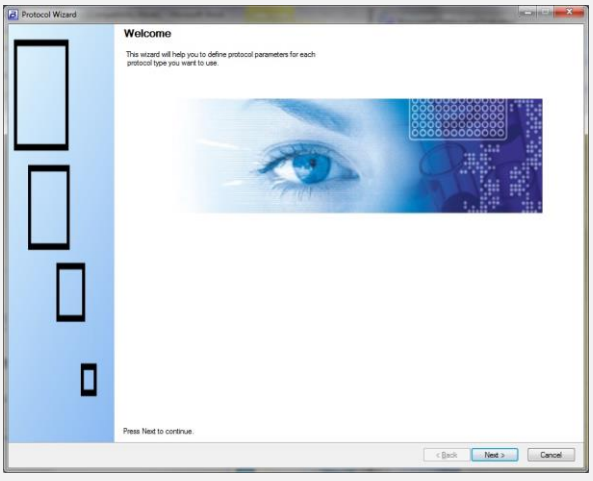
2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

   for editing an existing protocol use the **edit** icon

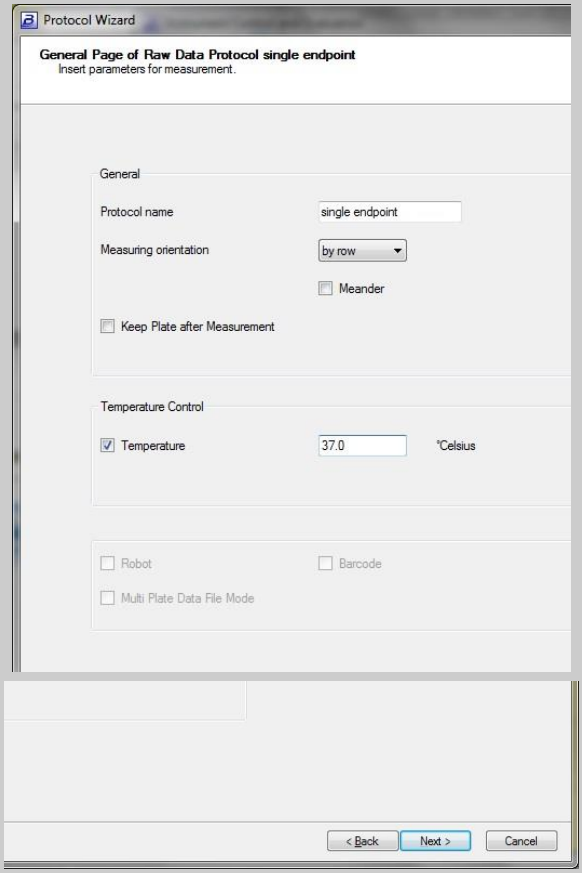


3. The start up screen of the protocol wizard will show up
   Click **<Next>**



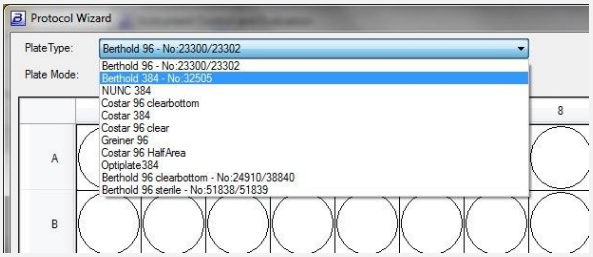
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



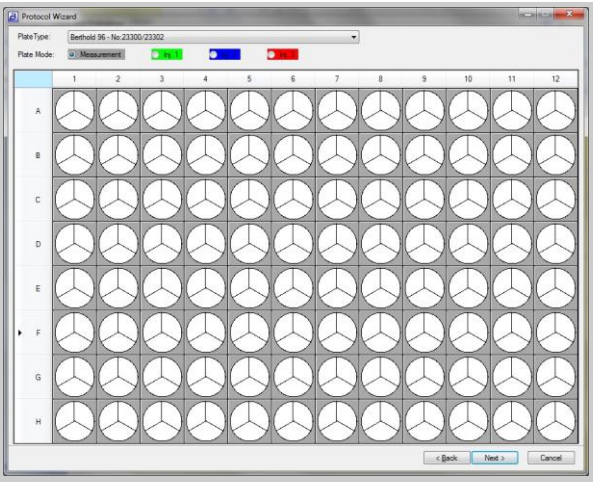
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

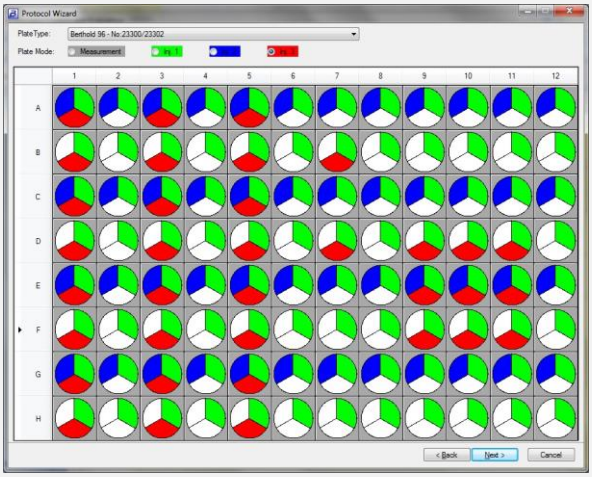
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

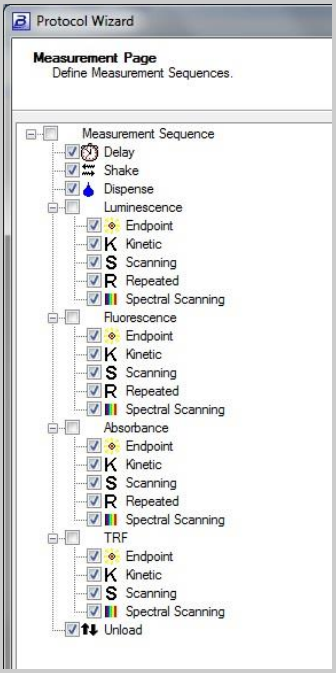
    Wells coloured in the respective colour are injected into

    Note: only wells to be measured can be injected into
14. Click **<Next>**



Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking **<OK>** selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well

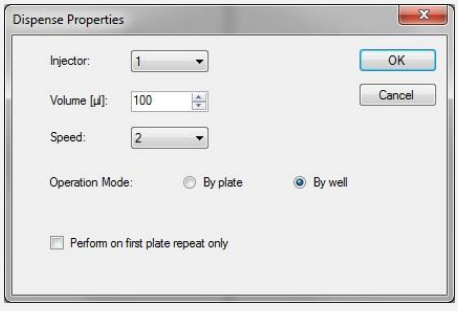


15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

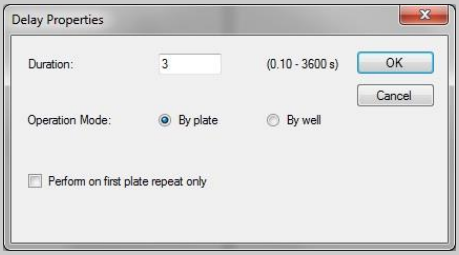
    In case additional reagent additions are required repeat this procedure for the other injector(s)



17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

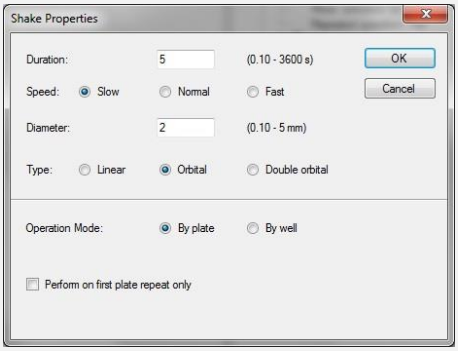
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Endpoint*** in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

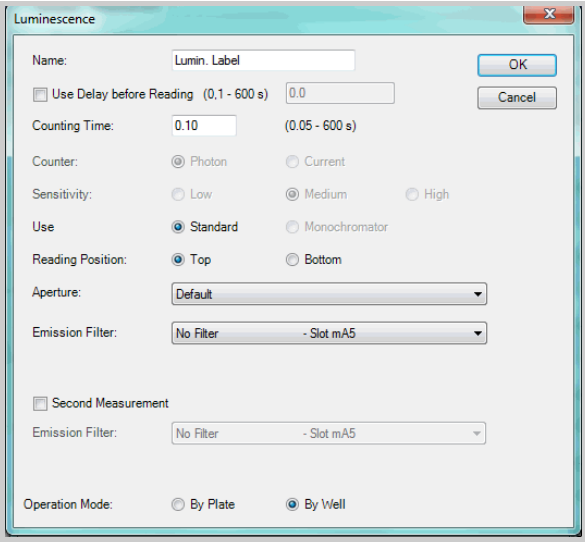
Note: filters must be defined prior in the Instrument menu

Reading Position	Choose reading from above (top) or below (Bottom) the plate. Usually: Top

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Operation Mode	by plate or by well

22. Click **<OK>**



21. Double-click ***Endpoint*** in the Fluorescence section for a fluorescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

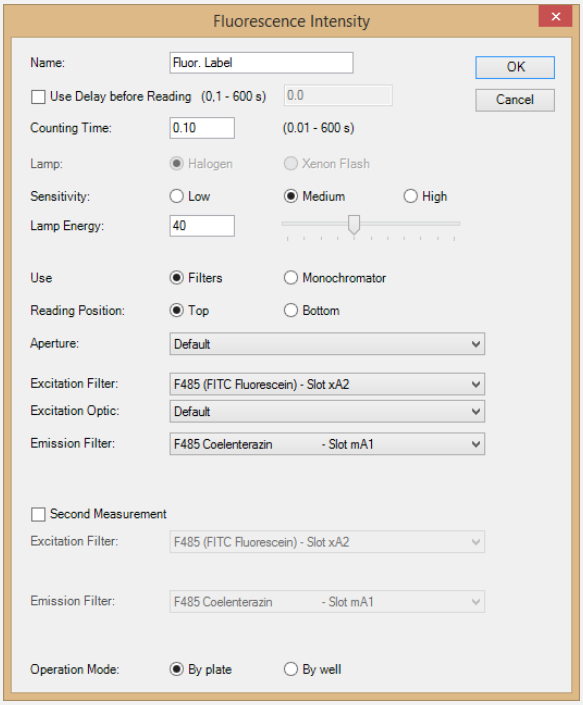
Note: filters must be defined prior in the Instrument menu

Reading Position	Choose reading from above (top) or below (Bottom) the plate

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used..

Operation Mode	by plate or by well

22. Click **<OK>**



21. Double-click ***Endpoint*** in the Absorbance section for an absorbance reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator
Measurem. Filter	select from the list

Note: The Aperture is recommended to be set to default. The Excitation optics are recommended to be set to *default, small* or *wide. Small* is especially recommended for UV-applications.

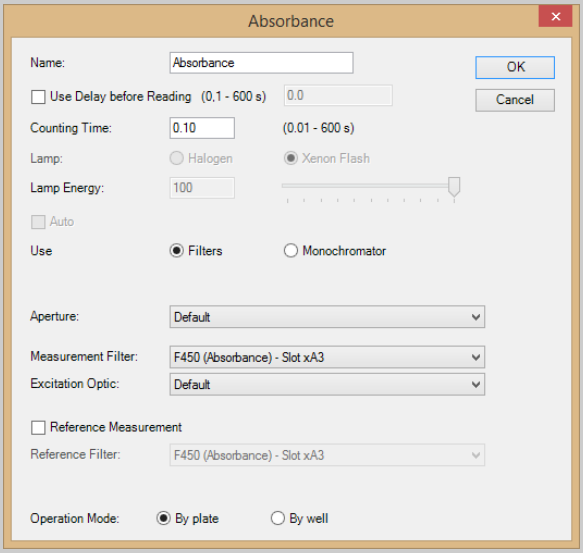
Meas. Wavelength	set value
Meas. Slit Width	set value

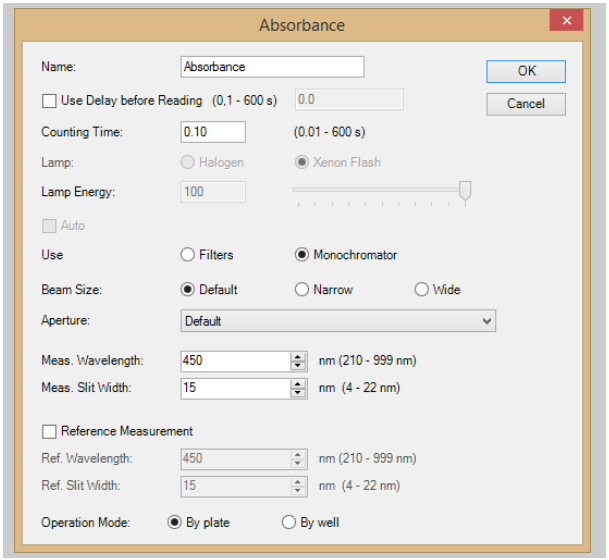
Reference Measurement

Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter	select from the list
Operation Mode	by plate or by well

22. Click **<OK>**





21. Double-click ***Endpoint*** in the TRF section for a time-resolved fluorescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator (for better sensitivity filters are recommended)
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

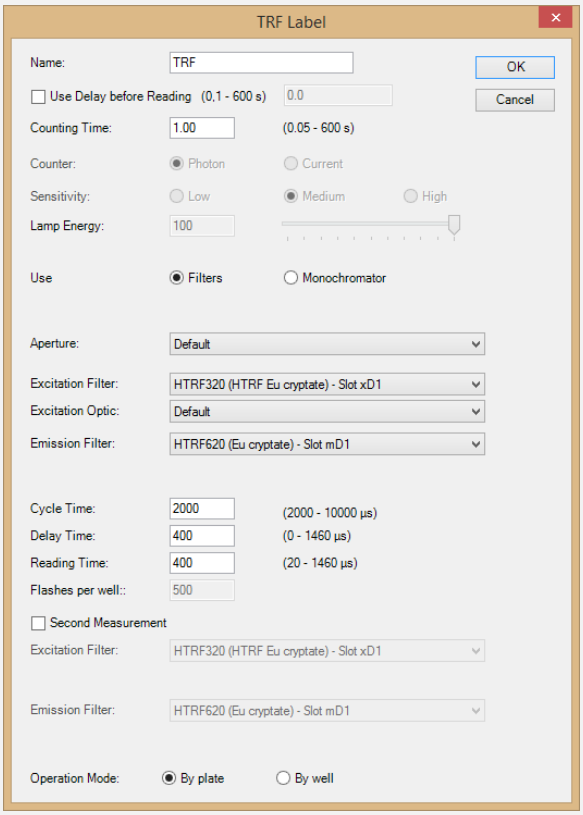
Cycle Time	set to 2000 µs repr. max. frequ. 500 Hz
Delay Time	time for which the detector is gated out, i.e. does not collect any signals (waiting for the unspecific prompt fluorescence to die off).

Typical settings are:

DELFIA® Europium	400 µs
DELFIA® Samarium	100 µs
DELFIA® Terbium	500 µs
HTRF®	100 µs (Filter)

Reading Time	time of the window within the PMT collects the time-resolved signal.

Typical settings are:



DELFIA® Europium	400 µs
DELFIA® Samarium	100 µs
DELFIA® Terbium	1400 µs
HTRF®	300 µs
Flashes per well	calculated
Operation Mode	by plate or by well

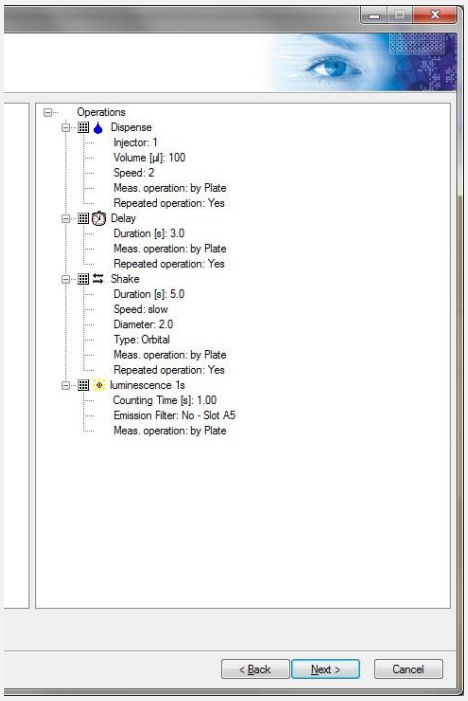
22. Click **<OK>**

23. The **sequence of selected operations** will be displayed on the right-hand side

    Operations can be moved up or down by clicking on the operation and dragging them to the respective position (***need to be collapsed***)

    Operations can be deleted by highlighting and hitting the **DEL** key or by dragging to the left

24. Click **<Next>**



25. Define **Export** settings

    Type your **Header** specific for this protocol

    Select the data set by dragging from left to right

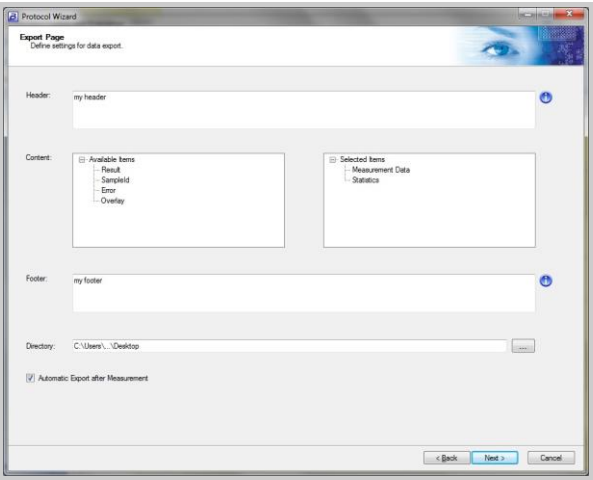
Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required

26. Click **<Next>**



27. Define **Print** settings

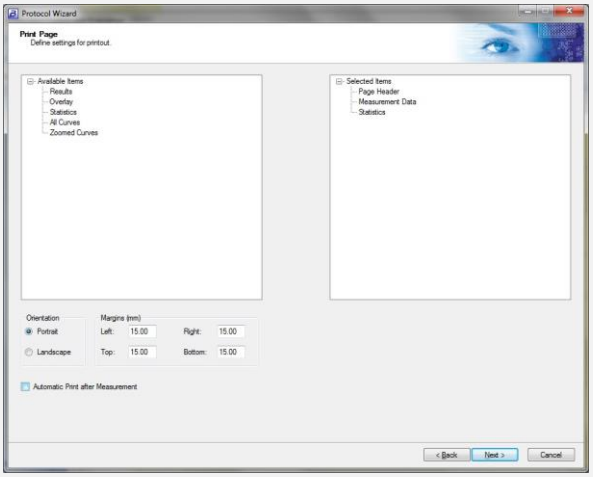
    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

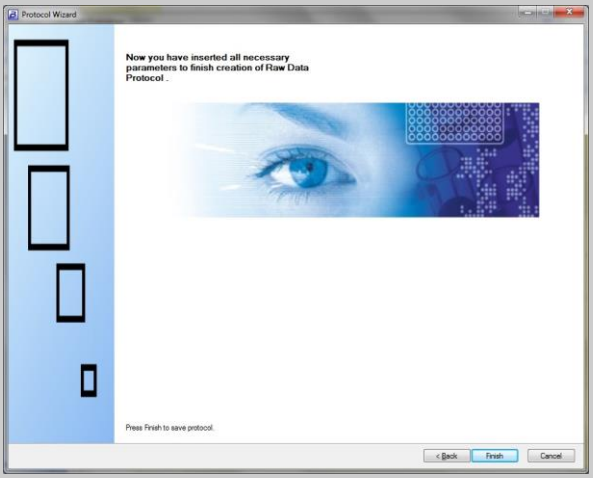
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

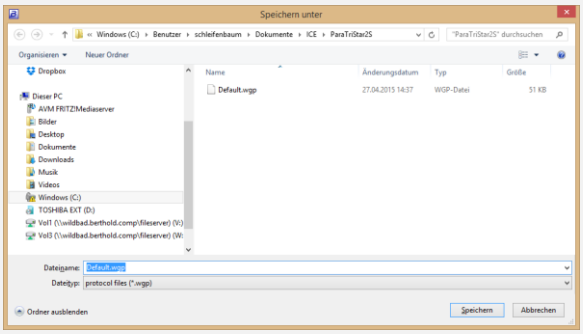
28. Click **<Next>**



29. Click **<Finish>**



30. Define the protocol **file name**
31. Click **<Save>**



7.2.2 Measurement with a Single Endpoint protocol

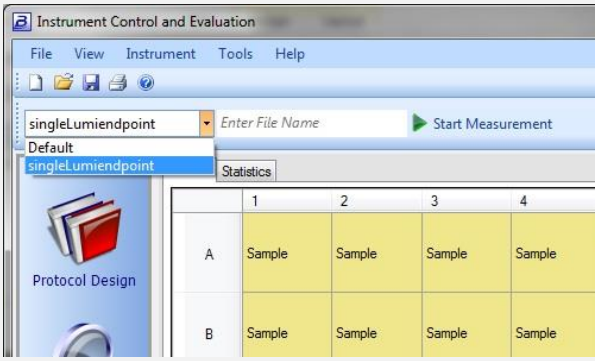
The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

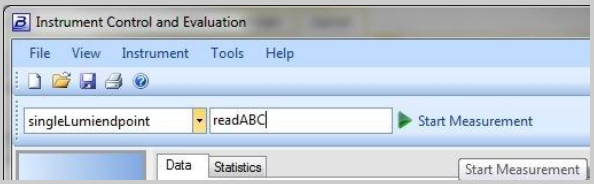
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

4. Insert the **microplate** with your samples:
well A1 facing the rear and left

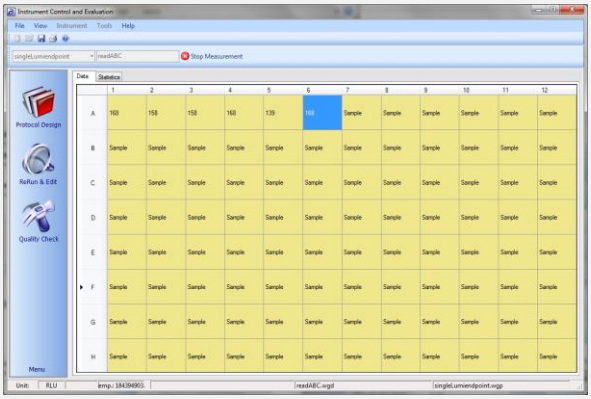
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

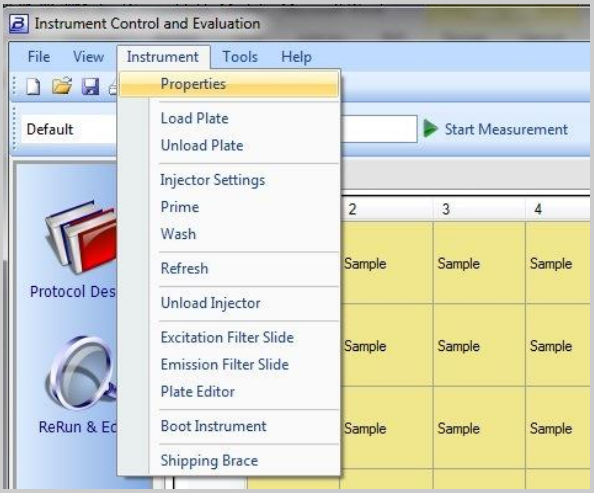
   Use the **green frame** for ***lidded*** microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

5. Click **<OK>**

6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed



7. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.3 Dual Label Assay measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine dual reporter gene expression.

7.3.1 Defining a Dual Label protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

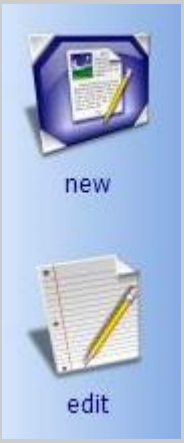
1. Click icon **Protocol Design** in the left-hand **Navigation** bar

   the navigation bar will appear in a new design

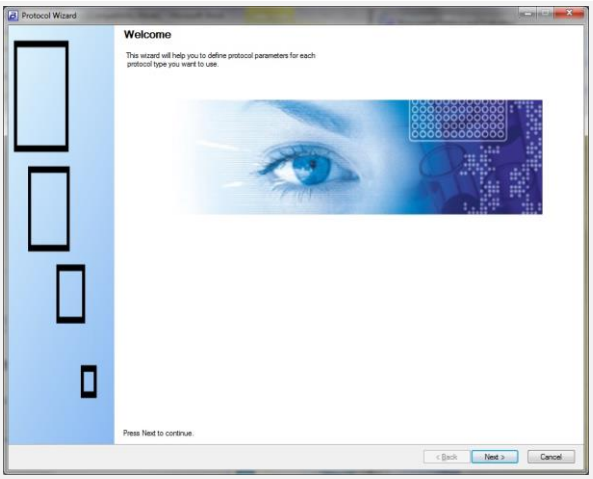
2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

   for editing an existing protocol use the **edit** icon

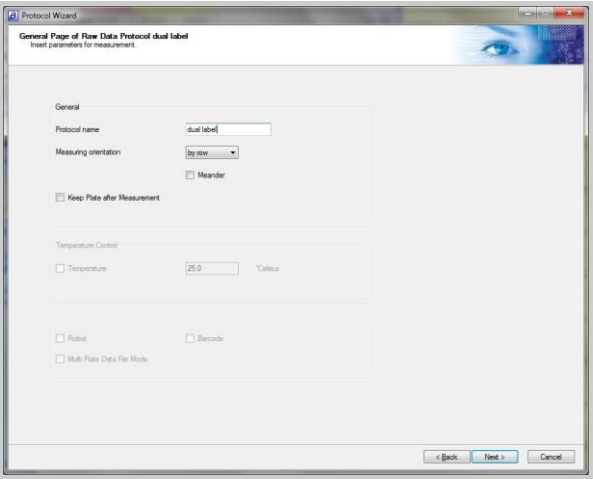


3. The start up screen of the protocol wizard will show up
   Click **<Next>**



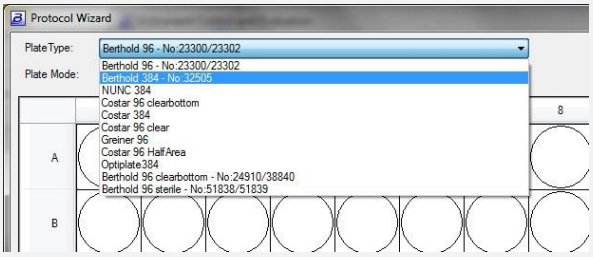
4. Enter a (descriptive) N**ame** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



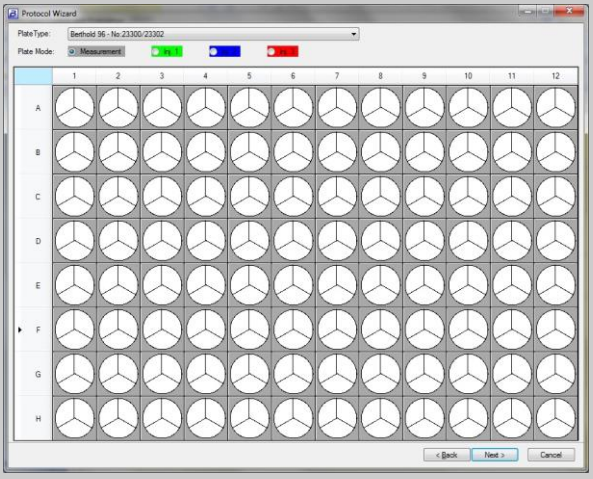
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

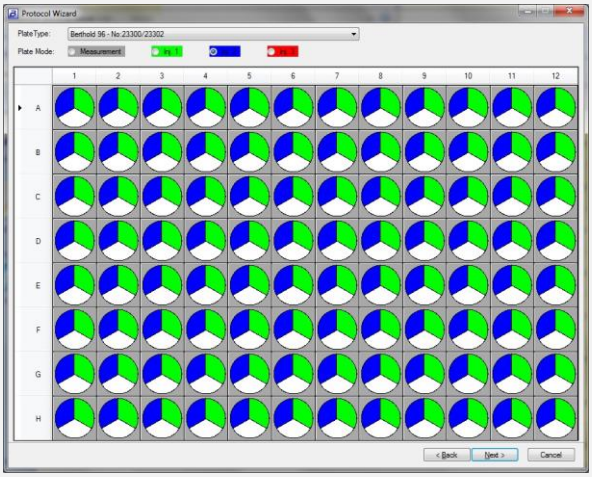
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

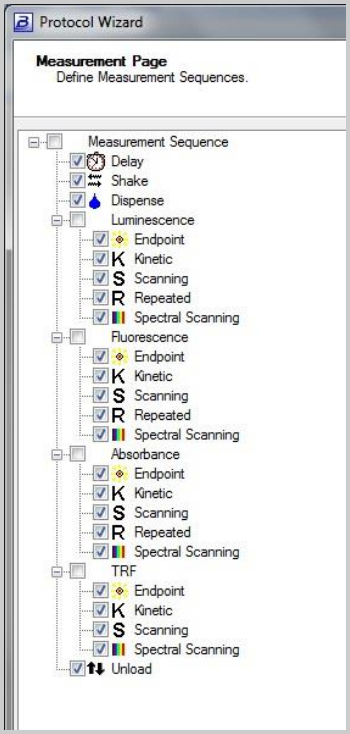
    Wells coloured in the respective colour are injected into

    Note: only wells to be measured can be injected into
14. Click **<Next>**



Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well

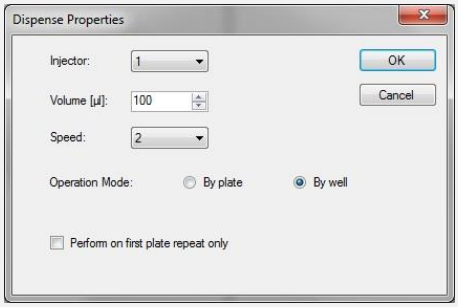


15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

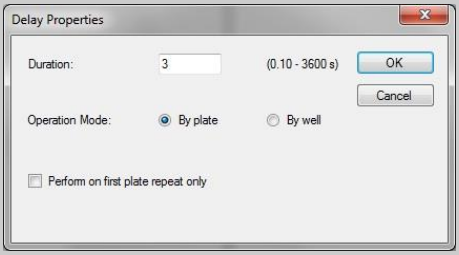
    In case additional reagent additions are required repeat this procedure for the other injector(s)



17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

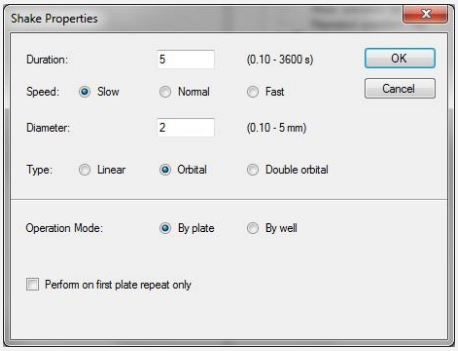
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Endpoint***, e.g. in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

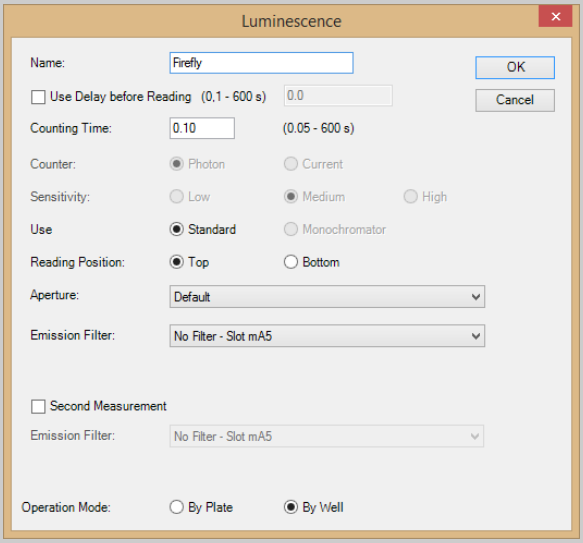
Note: filters must be defined prior in the Instrument menu

Reading Position	above (top) or below (Bottom) the plate. Usually: Top

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Operation Mode	by plate or by well

22. Click **<OK>**



23. Once more double-click ***Endpoint*** , e.g. in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

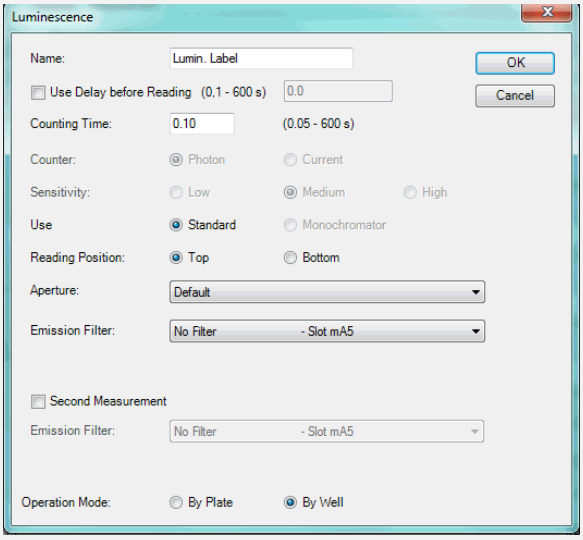
    Note: filters must be defined prior in the Instrument menu

Reading Position	above (top) or below (Bottom) the plate. Usually: Top

    Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Operation Mode	by plate or by well

24. Click **<OK>**

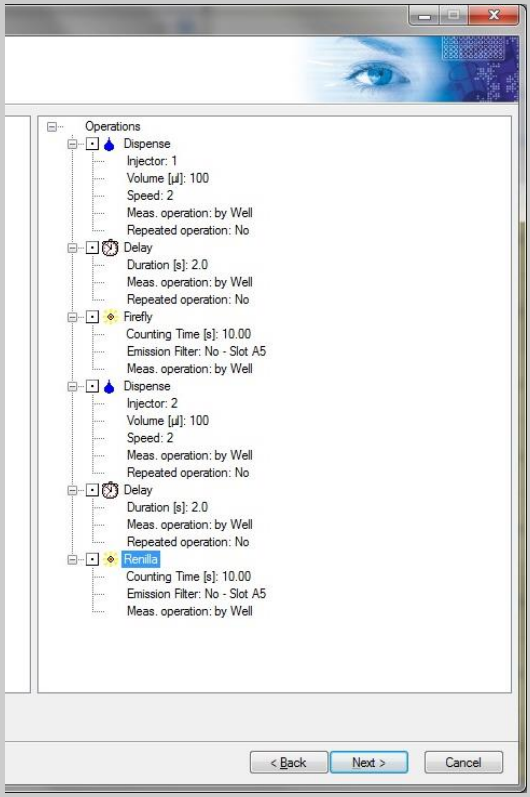


25. The **sequence of selected operations** will be displayed on the right-hand side

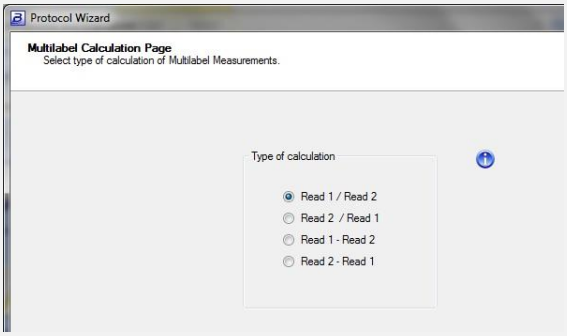
    Operations can be moved up or down by clicking on the operation and dragging them to the respective position

    Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

26. Click **<Next>**



27. Select the calculation to perform with the 2 measurements
28. Click **<Next>**



29. Define **Export** settings

    Type your **Header** specific for this protocol

    Select the data set by dragging from left to right

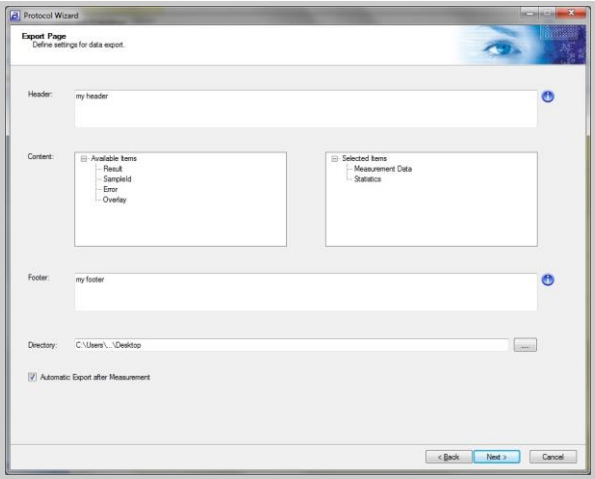
Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required

30. Click **<Next>**



31. Define **Print** settings

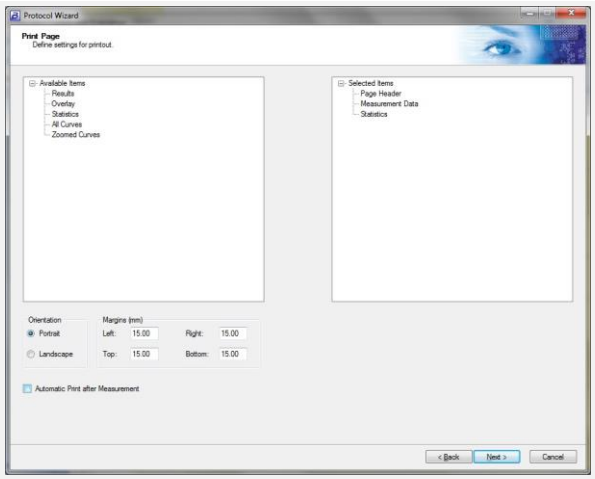
    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

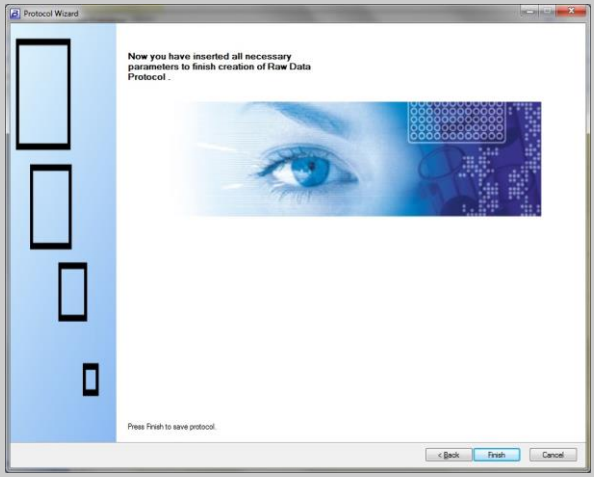
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

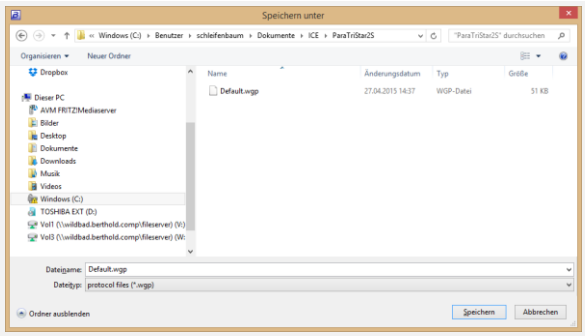
32. Click **<Next>**



33. Click **<Finish>**



34. Define the protocol **file name**
35. Click **<Save>**



7.3.2 Measurement with a Dual Label Assay protocol

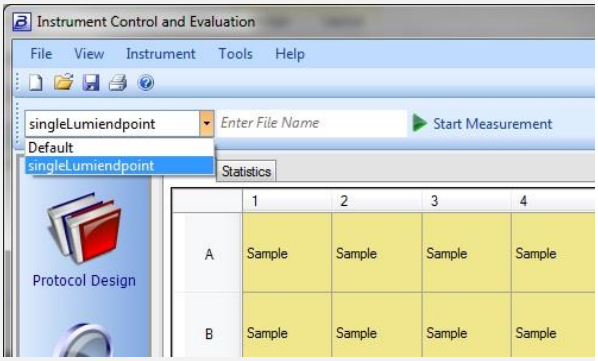
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

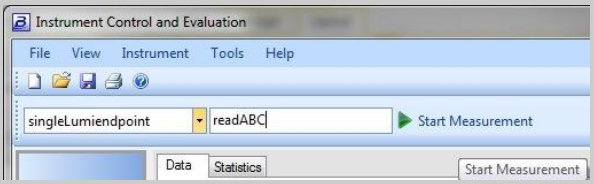
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

4. Insert the **microplate** with your samples:
well A1 facing the rear and left

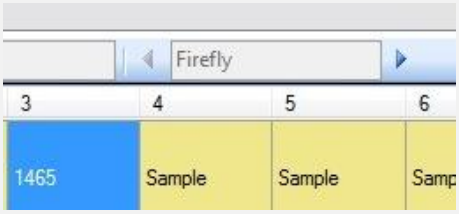
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

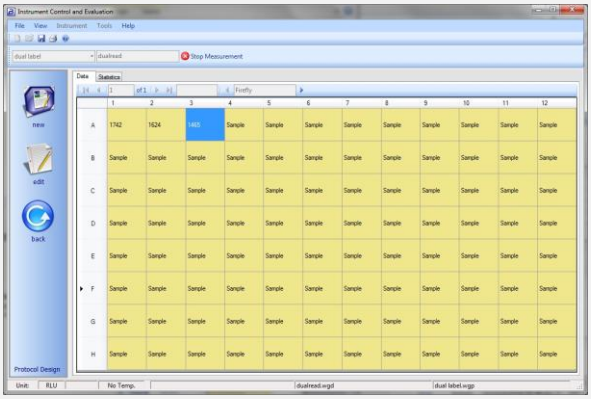
   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

5. Click **<OK>**

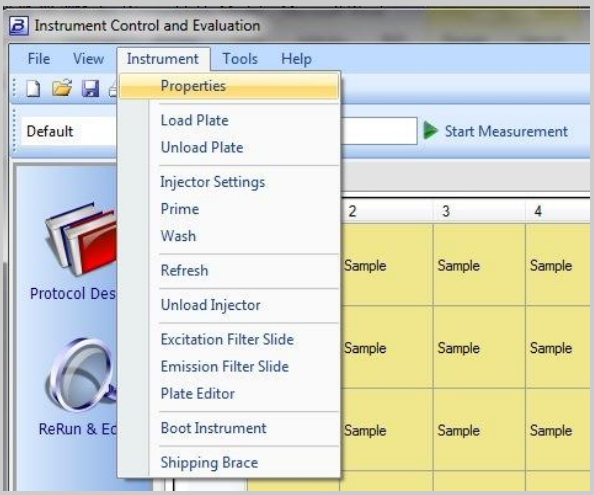
6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

   You may switch between the two readings by clicking on the arrows





7. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.4 Fluorescence Polarisation (FP) Assay measurement

Fluorescence Polarisation (FP) assays require only one fluorescent label to determine a molecular binding. To

7.4.1 Defining a Fluorescence Polarisation (FP) protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

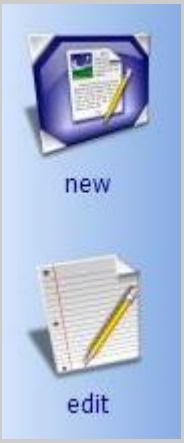
36. Click icon **Protocol Design** in the left-hand **Navigation** bar

    the navigation bar will appear in a new design

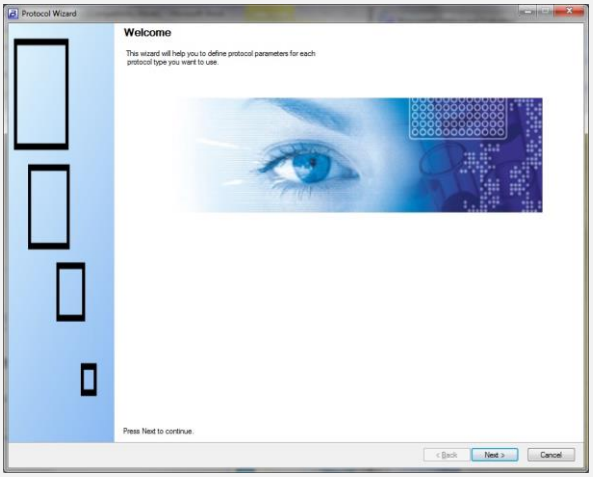
37. Click the **new** icon in the left-hand **Navigation** bar

    again, the navigation bar will appear in a new design

    for editing an existing protocol use the **edit** icon

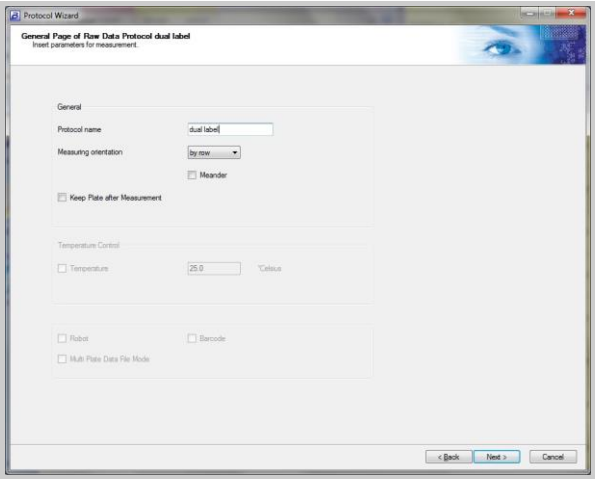


38. The start up screen of the protocol wizard will show up
    Click **<Next>**



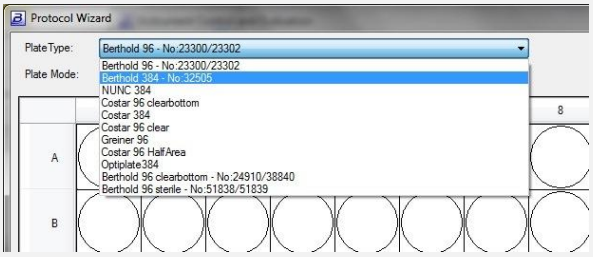
39. Enter a (descriptive) N**ame** for your protocol
40. Define the **reading orientation**:
    by column or by row
41. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
42. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
43. Check **Temperature** to activate the temperature control for this protocol
44. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active
45. Click **<Next>**



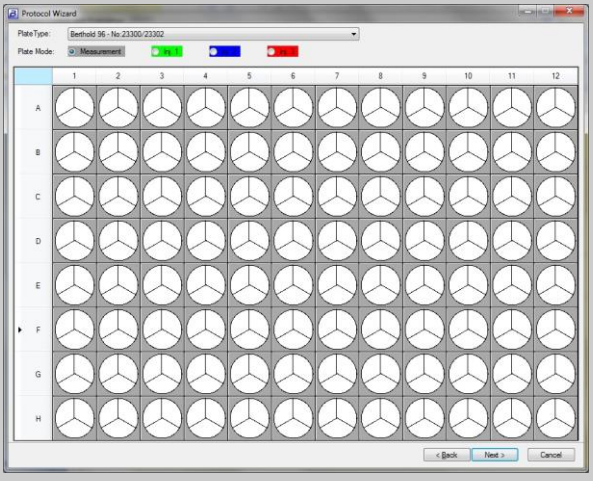
46. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



47. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

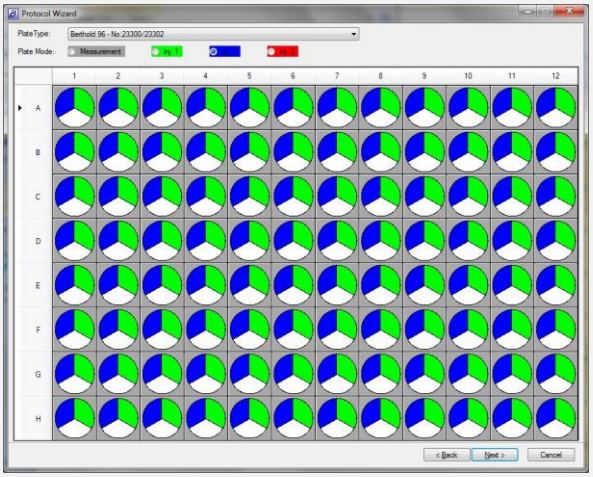
    Wells with a gray outside area are selected for measurement



48. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

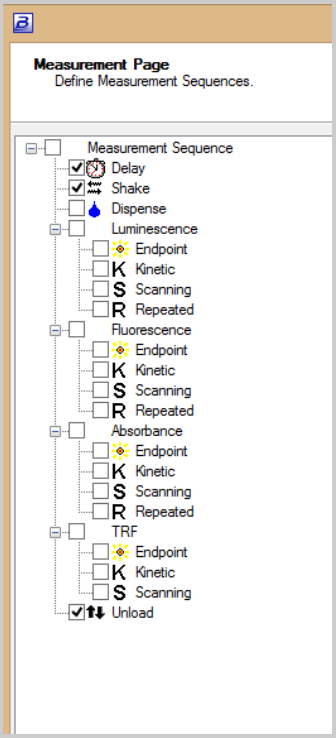
    Wells coloured in the respective colour are injected into

    Note: only wells to be measured can be injected into
49. Click **<Next>**



Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well

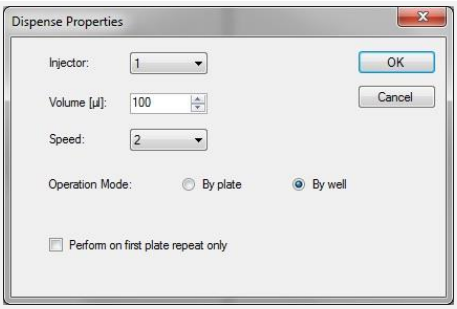


50. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

51. Click **<OK>**

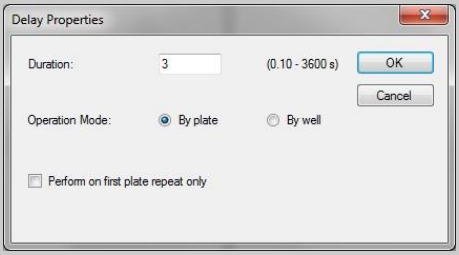
    In case additional reagent additions are required repeat this procedure for the other injector(s)



52. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

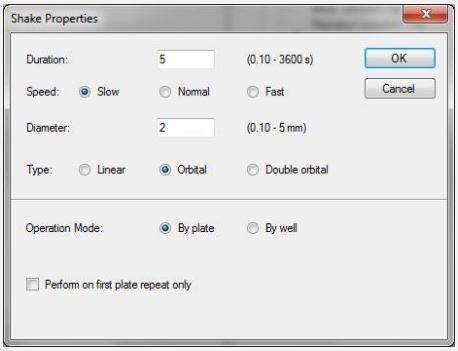
53. Click **<OK>**



54. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

55. Click **<OK>**



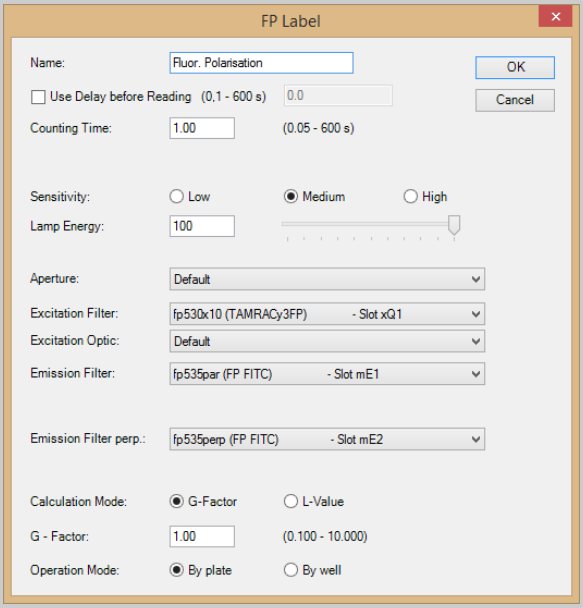
56. Double-click ***FP Label***

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Excitation Filter	choose the appropriate ex citation filter
Emission Filter	choose the appropriate emission filter for vertically oriented fluorescence
Emission Filter perp.	choose the appropriate emission filter for perpen dicularly oriented fluores cence

Note: FP filters use precisely aligned polarisation filters. For ideal results only use special FP fiter slides (

Note: filters must be defined prior in the Instrument menu

G-Factor	Enter the correct G factor for your assay and this in strument derived from a G factor determination meas urement.
L-Value	Enter the correct L value for your assay and this in strument derived from a L value determination meas urement.
Operation Mode	by plate or by well



57. Click **<OK>**

58. Define **Export** settings

    Type your **Header** specific for this protocol

    Select the data set by dragging from left to right

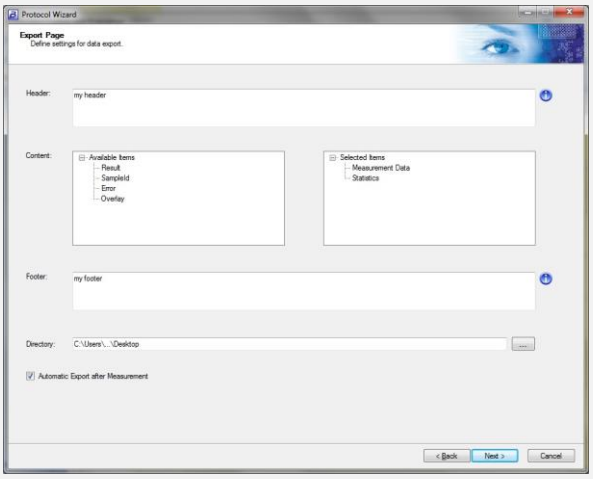
Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required

59. Click **<Next>**



60. Define **Print** settings

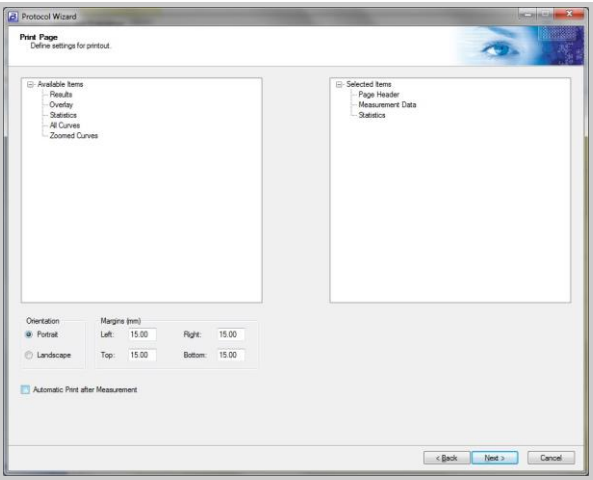
    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

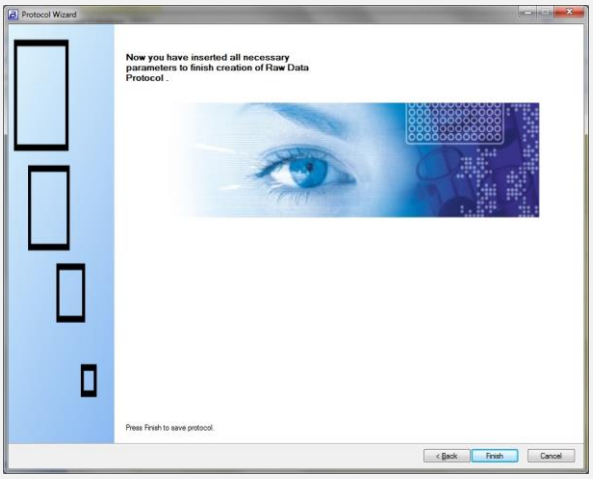
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

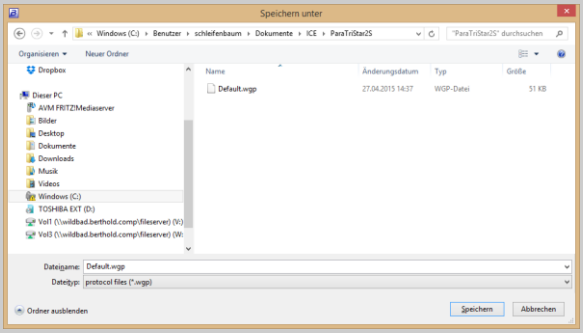
61. Click **<Next>**



62. Click **<Finish>**



63. Define the protocol **file name**
64. Click **<Save>**



7.4.2 Measurement with a Fluorescence Polarisation (FP) Assay protocol

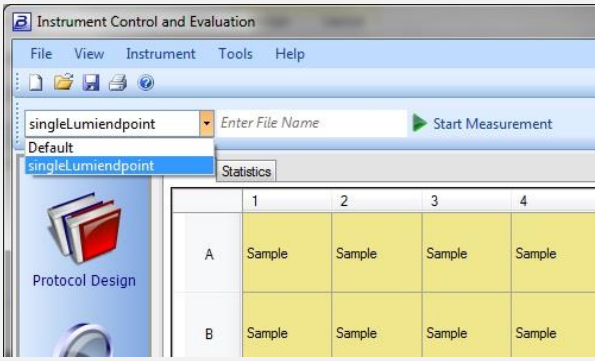
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

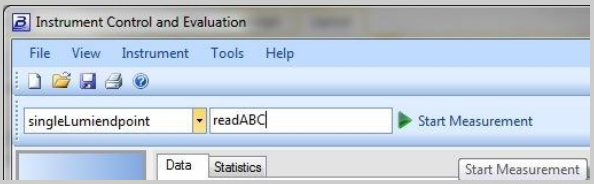
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

8. Select the **protocol** to be used



9. Enter a **file name** under which the measurement is to be stored



10. Click **<Start Measurement>**

11. Insert the **microplate** with your samples:
well A1 facing the rear and left

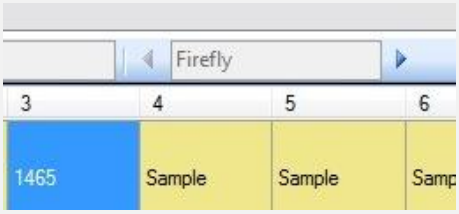
    Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

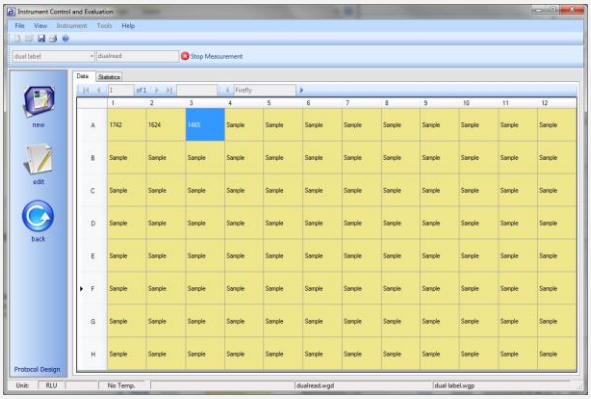
    Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

12. Click **<OK>**

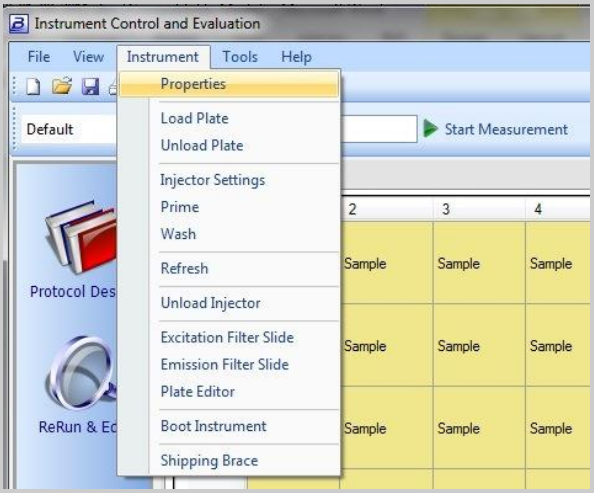
13. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

    You may switch between the two readings by clicking on the arrows





14. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.5 Kinetic Measurement

A kinetic measurement mode is appropriate for fast kinetics assays lasting over several seconds up to minutes, e.g. enzyme kinetics and Calcium influx

7.5.1 Defining a protocol for a kinetic measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

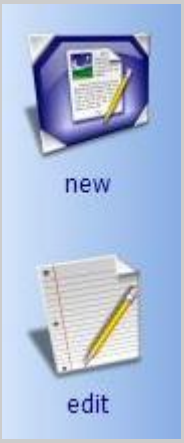
1. Click icon **Protocol Design** in the left-hand **Navigation** bar

   the navigation bar will appear in a new design

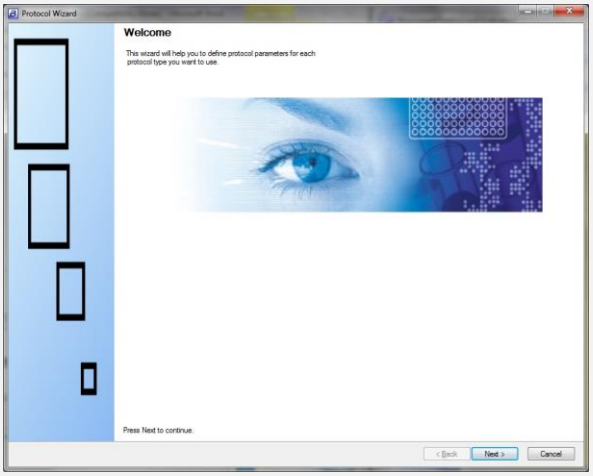
2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

   for editing an existing protocol use the **edit** icon

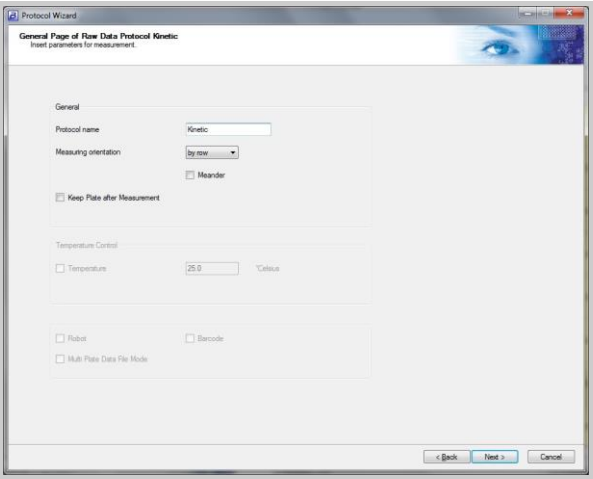


3. The start up screen of the protocol wizard will show up
   Click **<Next>**



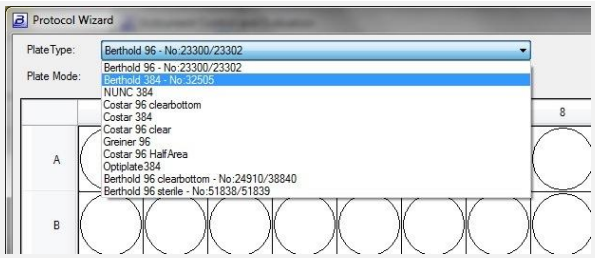
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



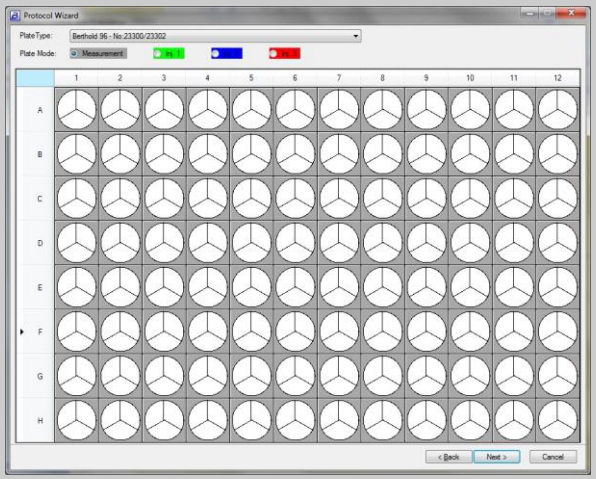
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

    Wells with a gray outside area are selected for measurement

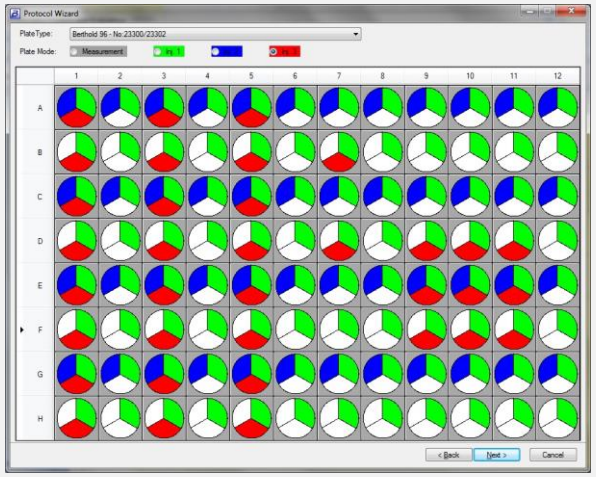


13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

    Wells coloured in the respective colour are injected into

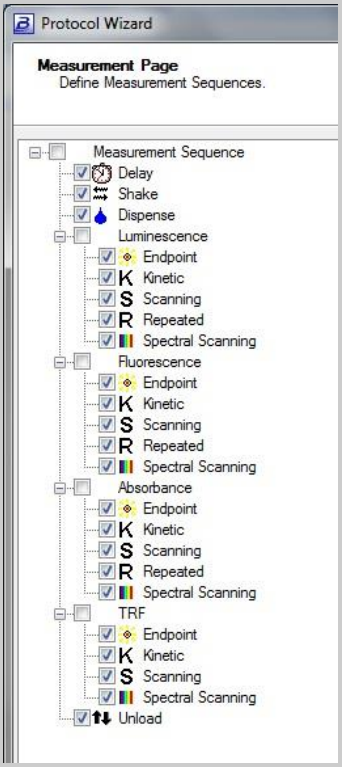
    Note: only wells to be measured can be injected into

14. Click **<Next>**



Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well

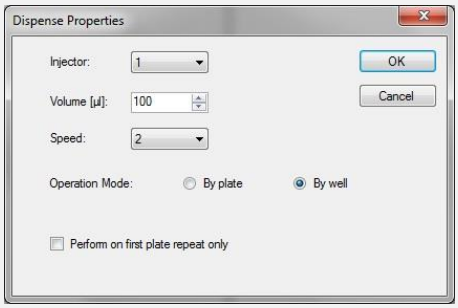


15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

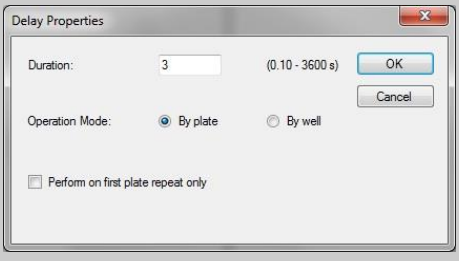
In case additional reagent additions are required repeat this procedure for the other injector(s)



17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

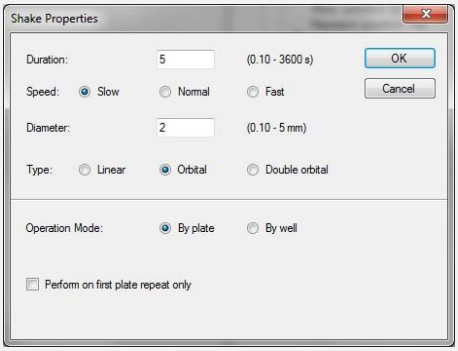
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Kinetic*** in the Luminescence section for a luminescence kinetic reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay	0 to 600 sec
Repeats	(are calculated)
Reading Position	Choose reading from above (top) or below (Bot tom) the plate.

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

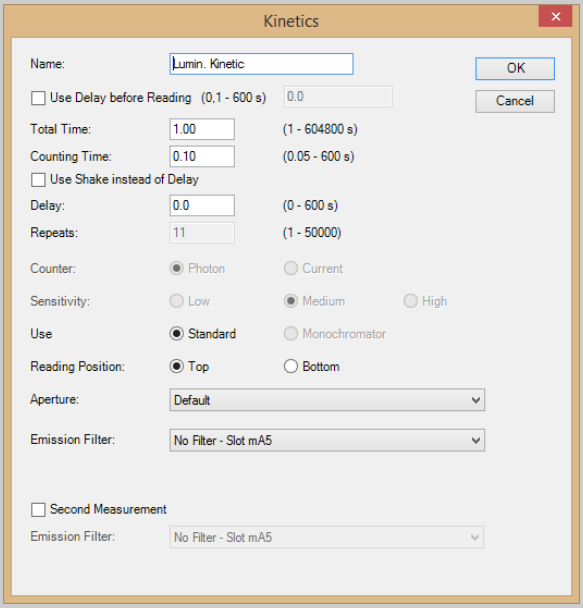
Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

Second Measurement may be checked in case of ratiometric kinetics, e.g. in BRET applications

22. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way



23. Double-click ***Kinetic*** in the Fluorescence section for a fluorescence kinetic reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay	0 to 600 s
Repeats	(are calculated)
Reading Position	Choose reading from above (top) or below (Bot tom) the plate.

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

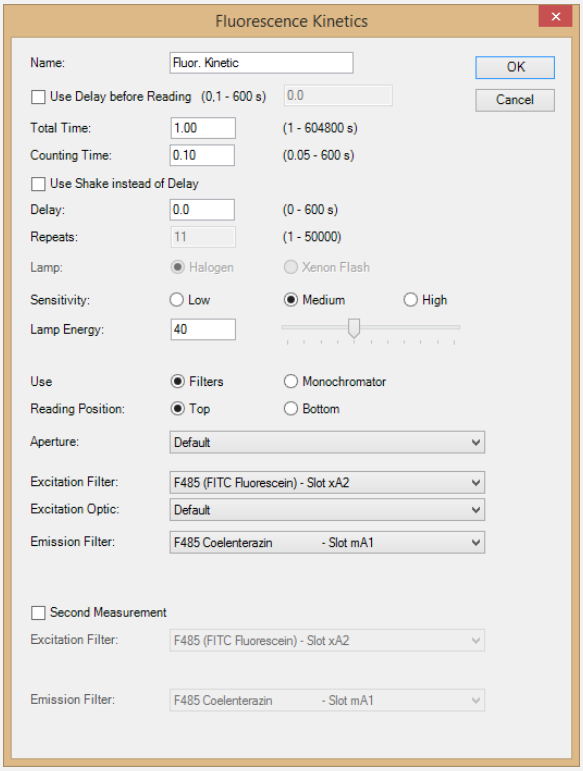
Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well

Second Measurement may be checked in case of ratiometric kinetics, e.g. in Calcium applications

24. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way



25. Double-click ***FP Kinetic*** in the Fluorescence section for a fluorescence polarisation kinetic reading

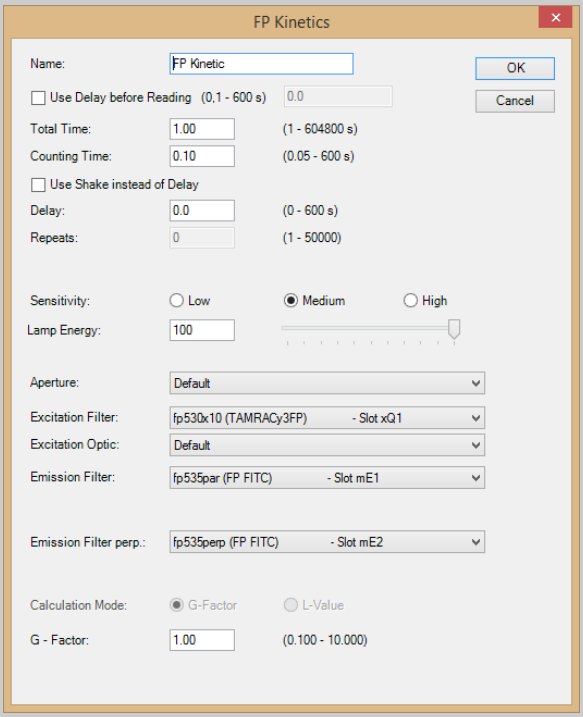
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Check Use Shake instead of Delay if needed	
Delay	0 to 600 s
Repeats	(are calculated)
Excitation Filter	choose the appropriate ex citation filter
Emission Filter	choose the appropriate emission filter for vertically oriented fluorescence
Emission Filter perp.	choose the appropriate emission filter for perpen dicularly oriented fluores cence

Note: FP filters use precisely aligned polarisation filters. For ideal results only use special FP fiter slides

Note: filters must be defined prior in the Instrument menu

G-Factor	Enter the correct G factor for your assay and this in strument derived from a G factor determination meas urement.
L-Value	Enter the correct L value for your assay and this in strument derived from a L value determination meas urement.
Operation Mode	by plate or by well

1.



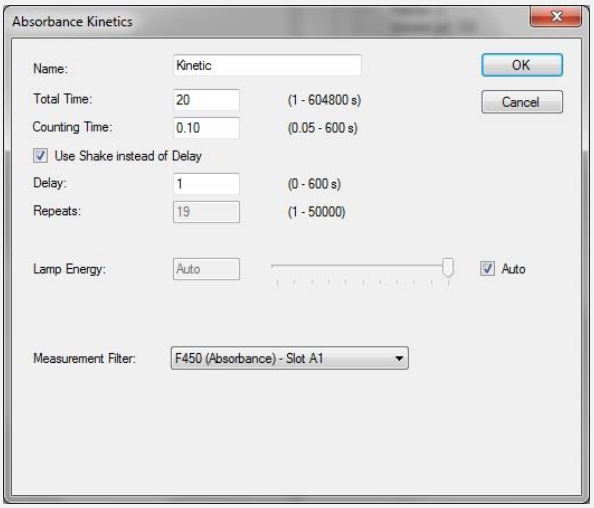
2. Double-click ***Kinetic*** in the Absorbance section for an absorbance kinetic reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake instead of Delay if needed	
Delay	0 to 600 s
Repeats	(are calculated)
Use	Filters or Monochromator
Measurem. Filter	select from the list
Meas. Wavelength	set value
Meas. Slit Width	set value

   Note: filters must be defined prior in the Instrument menu

3. Click **<OK>**

   a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

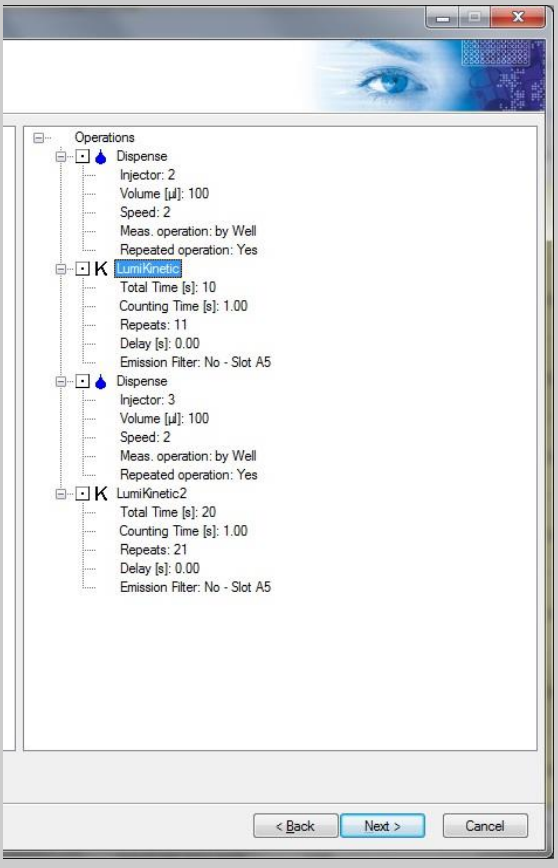


4. The **sequence of selected operations** will be displayed on the right-hand side

   Operations can be moved up or down by clicking on the operation and dragging them to the respective position

   Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

5. Click **<Next>**



6. Define **Export** settings

   Type your **Header** specific for this protocol

   Select the data set by dragging from left to right

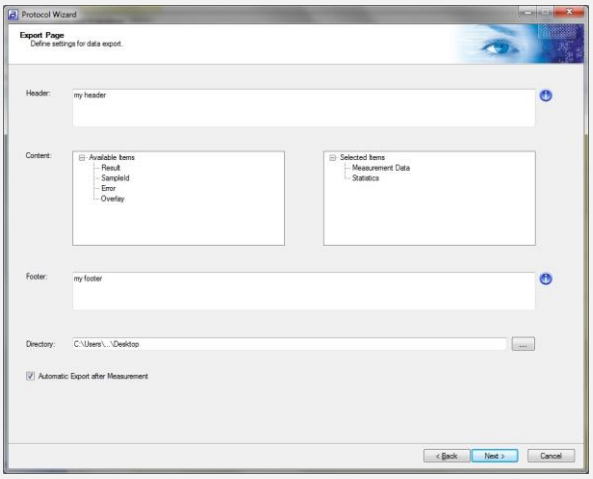
Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

   Type your **Footer** specific for this protocol

   Define **Directory** for the export file

   Check if **Automatic Export** is required

7. Click **<Next>**



8. Define **Print** settings

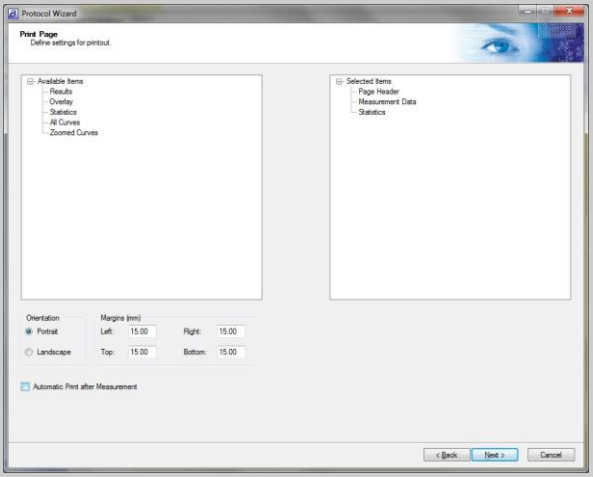
   Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

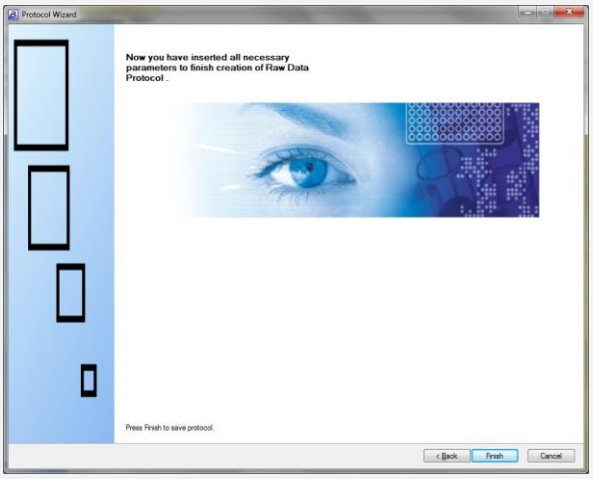
   Define **page orientation** and **margins**

   Check if **Automatic Print-out** is required

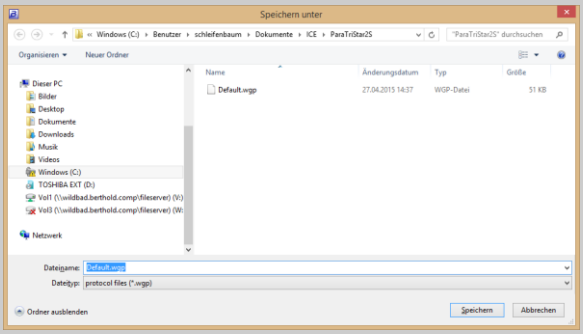
9. Click **<Next>**



10. Click **<Finish>**



11. Define the protocol **file name**
12. Click **<Save>**



7.5.2 Kinetic measurement

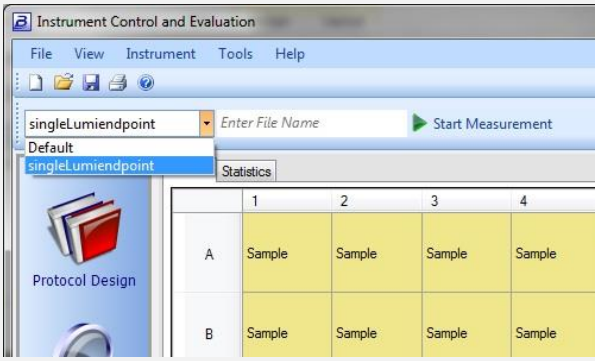
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

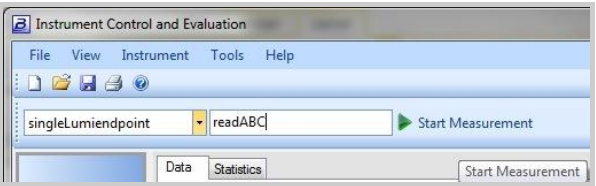
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

4. Insert the **microplate** with your samples:
well A1 facing the rear and left

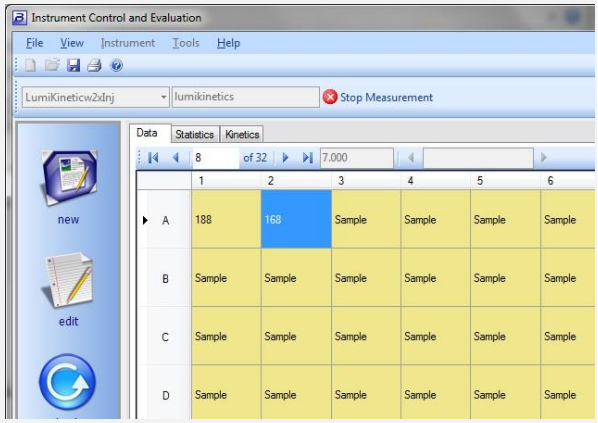
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

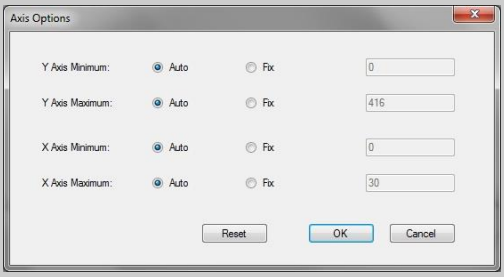
5. Click **<OK>**

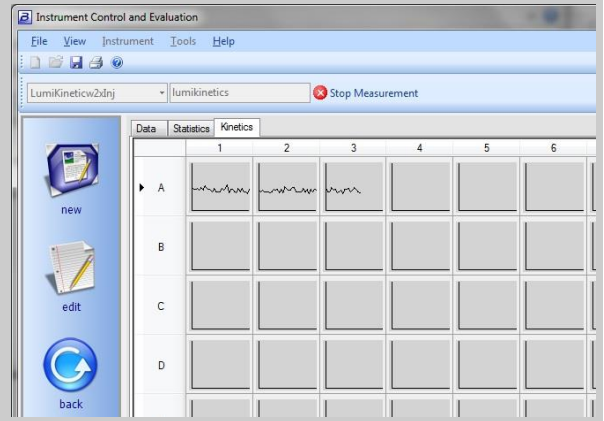
6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

   You may switch between the individual readings by clicking on the arrows

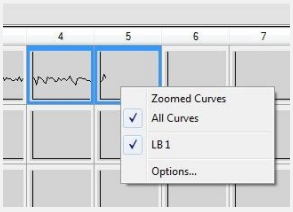


7. You also choose to view the curves by clicking the ***Kinetics*** tab
8. The scale of the axes can be changed by right-clicking into the curves and selecting **Options…**

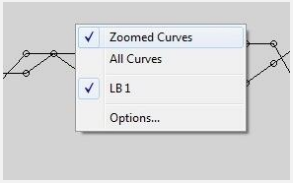


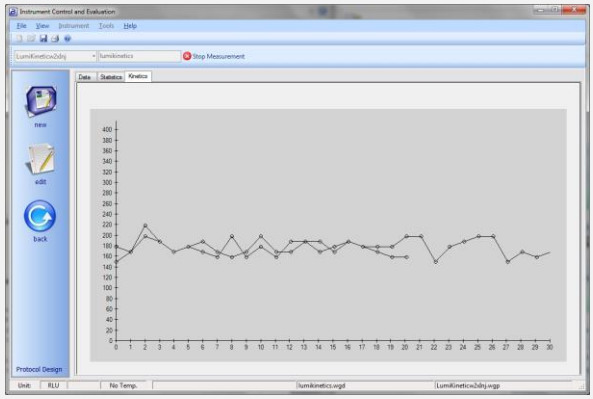


9. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Curves**

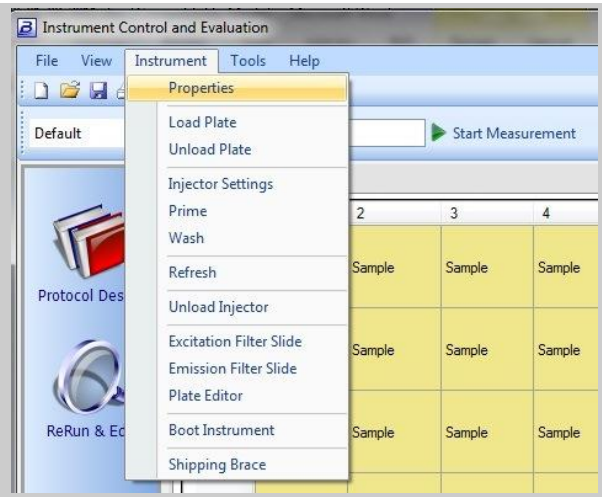


To un-zoom right-click into the zoomed view and select All Curves





10. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.6 Repeated Measurement

A repeated measurement mode is appropriate for long-term kinetic assays lasting over multiple minutes up to several days, e.g. cellular luminescence, slow enzyme kinetics, long-term gene expression or growth monitoring

7.6.1 Defining a protocol for a repeated measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

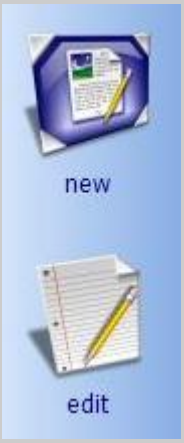
1. Click icon **Protocol Design** in the left-hand **Navigation** bar

   the navigation bar will appear in a new design

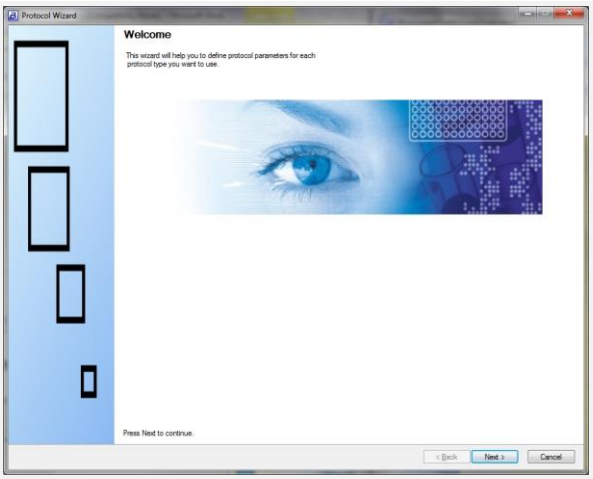
2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

   for editing an existing protocol use the **edit** icon

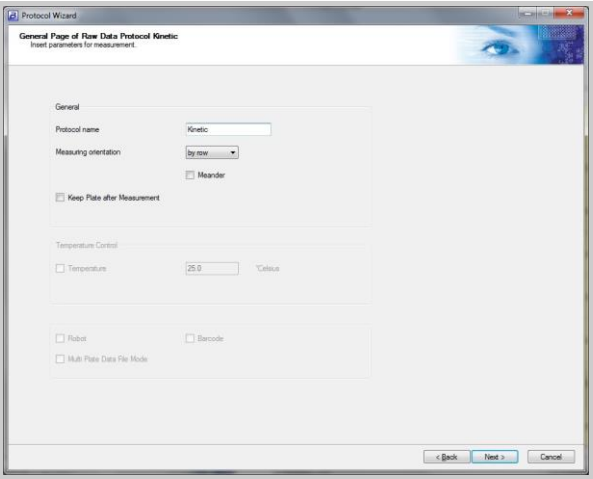


3. The start up screen of the protocol wizard will show up
   Click **<Next>**



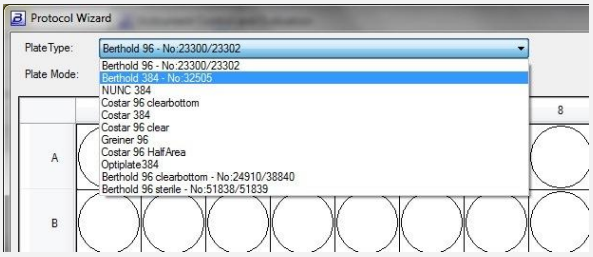
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



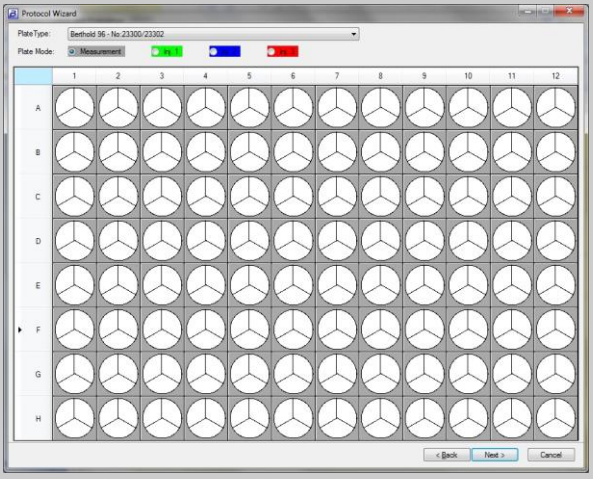
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

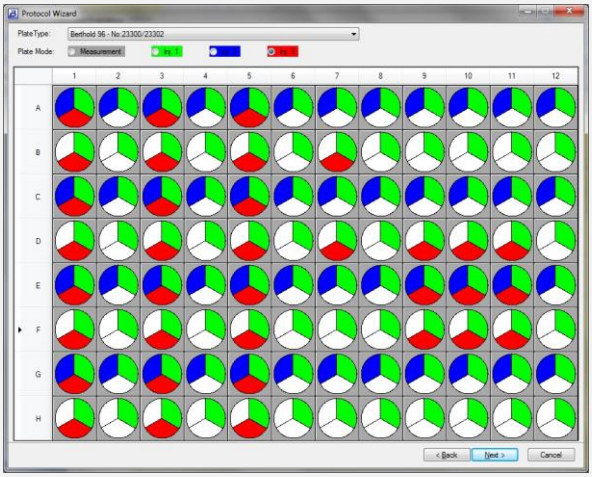
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

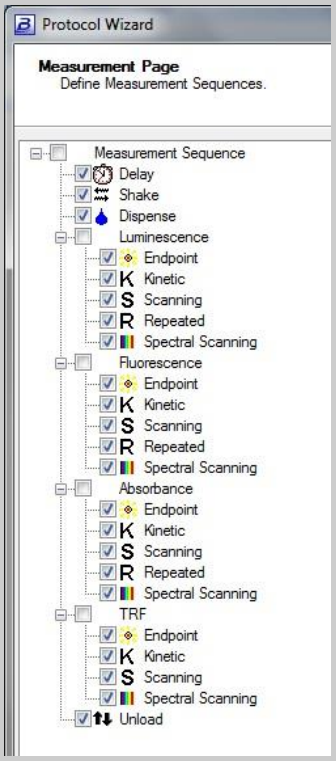
    Wells coloured in the respective colour are injected into

    Note: only wells to be measured can be injected into
14. Click **<Next>**



Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well

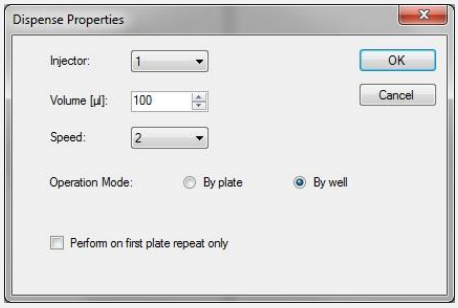


15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

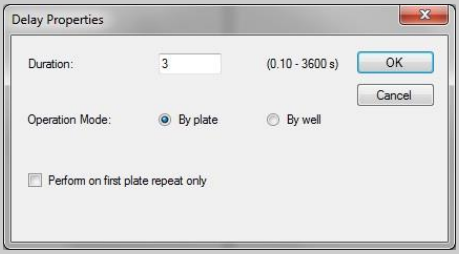
    In case additional reagent additions are required repeat this procedure for the other injector(s)



17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

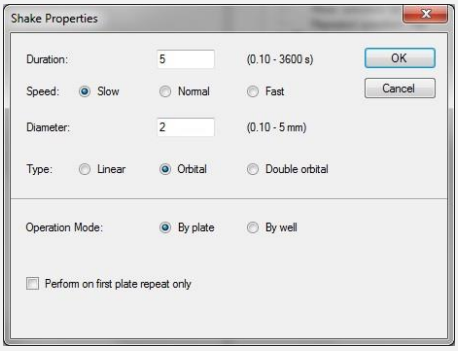
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Repeated*** in the Luminescence section for a luminescence repeated reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Emission Filter	usually: No Filter

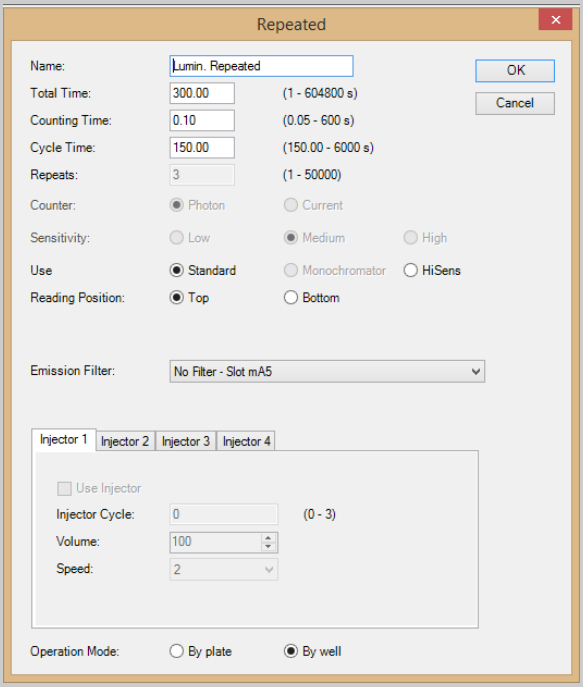
Note: filters must be defined prior in the Instrument menu

Reading Position	Choose reading from above (top) or below (Bot tom) the plate.

Note: If reading position ***Bottom*** is selected, the red plate frame must be used.

Injector 1, …2, …3

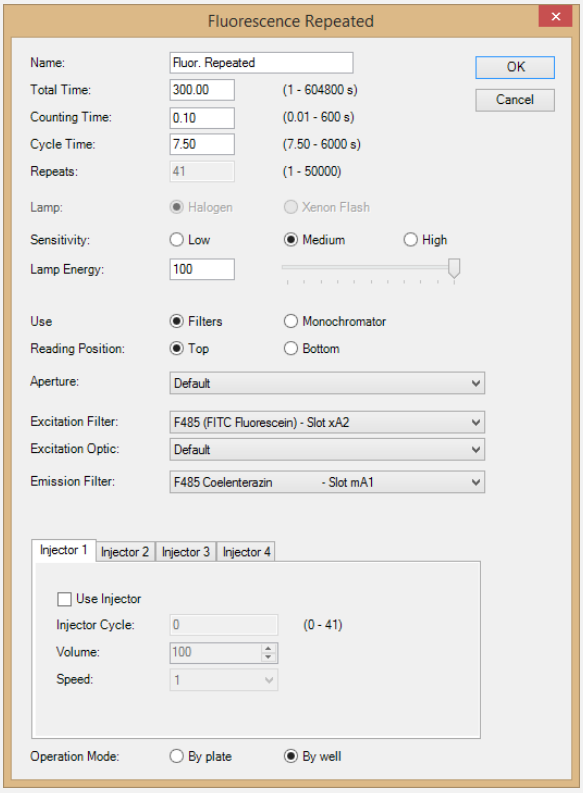
Check Use Injector for an injection within the repeated cycle	
Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well



22. Click **<OK>**

    a second repeated operation may be added, e.g. for ratiometric applications (BRET)

23. Double-click ***Repeated*** in the Fluorescence section for a fluorescence repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

    Note: filters must be defined prior in the Instrument menu

Reading Position	Choose reading from above (top) or below (Bot tom) the plate.

    Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

    Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle	
Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

24. Click **<OK>**

    a second repeated operation may be added, e.g. for ratiometric applications (FRET)

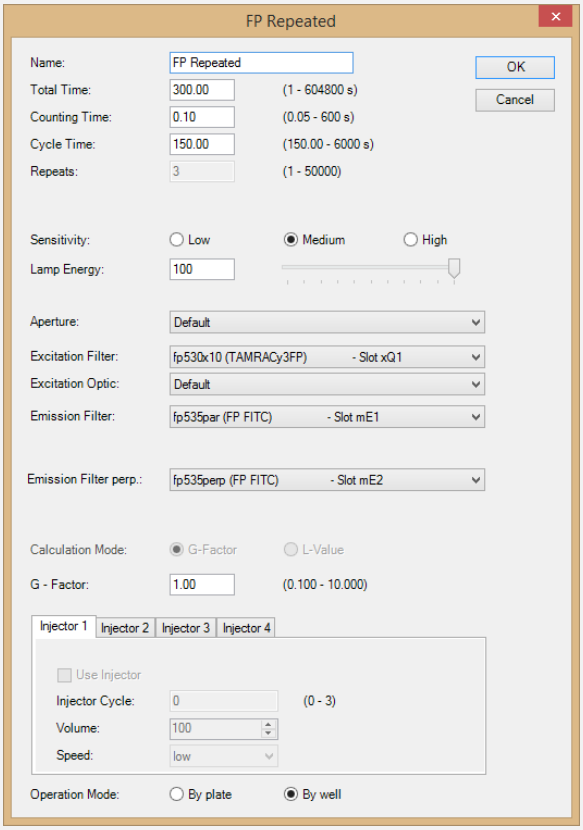
25. Double-click ***FP Repeated*** in the Fluorescence section for a fluorescence repeated reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Excitation Filter	choose the appropriate ex citation filter
Emission Filter	choose the appropriate emission filter for vertically oriented fluorescence
Emission Filter perp.	choose the appropriate emission filter for perpendicularly oriented fluorescence

Note: FP filters use precisely aligned polarisation filters. For ideal results only use special FP fiter slides

Note: filters must be defined prior in the Instrument menu

G-Factor	Enter the correct G factor for your assay and this instrument derived from a G factor determination measurement.
L-Value	Enter the correct L value for your assay and this instrument derived from a L value determination measurement.
Operation Mode	by plate or by well



26. Double-click ***Repeated*** in the Absorbance section for a absorbance repeated reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Use	Filters or Monochromator
Measurem. Filter	select from the list
Meas. Wavelength	set value
Meas. Slit Width	set value

Check Reference Measurement if needed

Reference Filter	select from the list
Meas. Wavelength	set value
Meas. Slit Width	set value

Note: filters must be defined prior in the Instrument menu

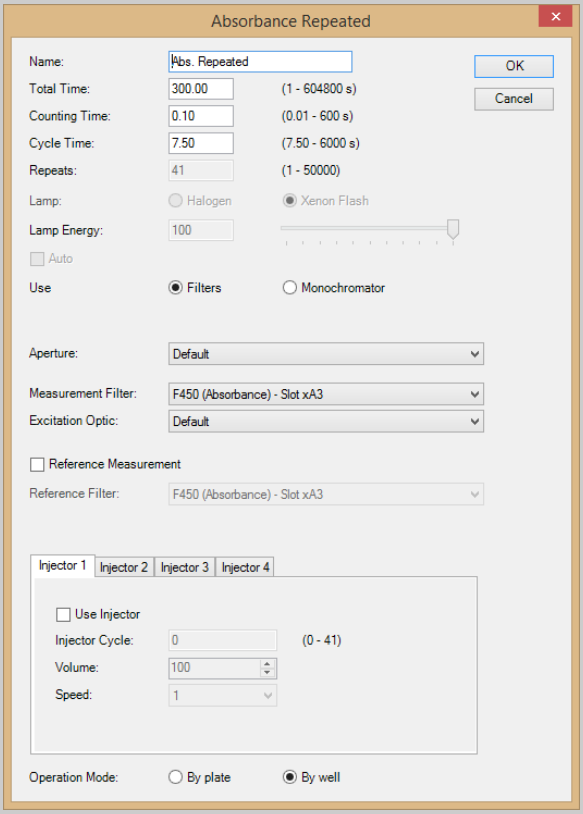
Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle

Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

27. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications

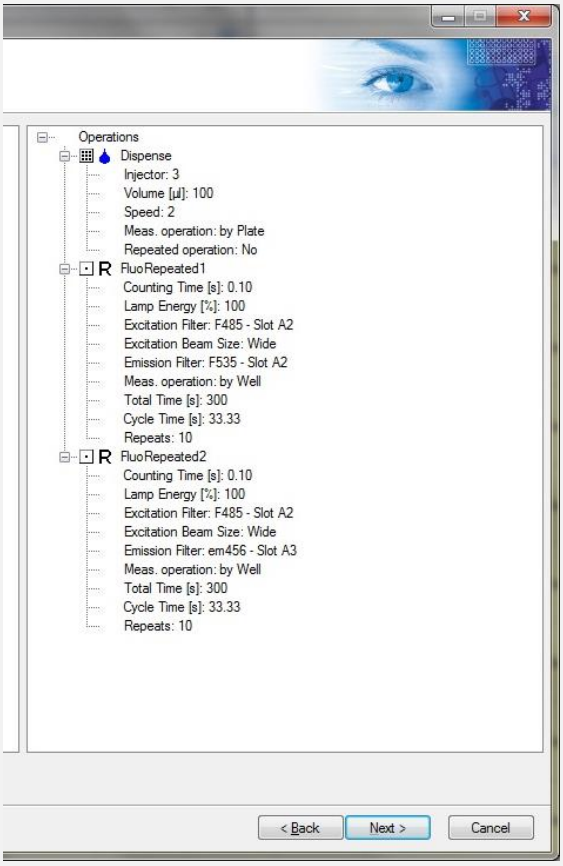


28. The **sequence of selected operations** will be displayed on the right-hand side

    Operations can be moved up or down by clicking on the operation and dragging them to the respective position

    Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

29. **Click <Next>**



30. Define **Export** settings

    Type your **Header** specific for this protocol

    Select the data set by dragging from left to right

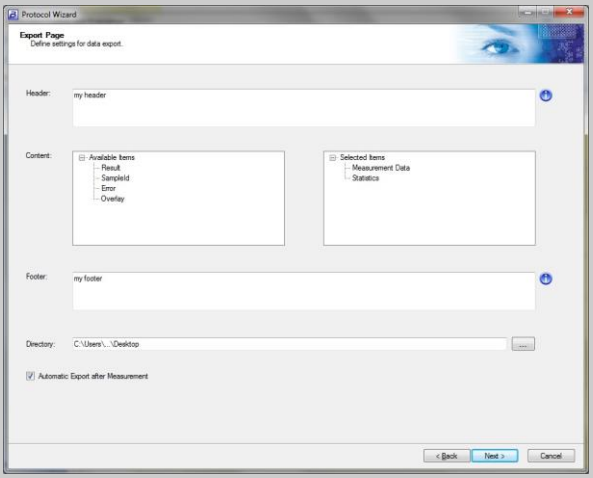
Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required

31. **Click <Next>**



32. **Define Print** settings

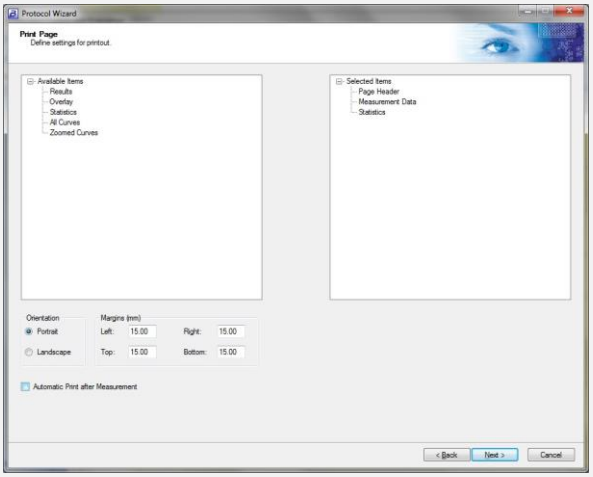
    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

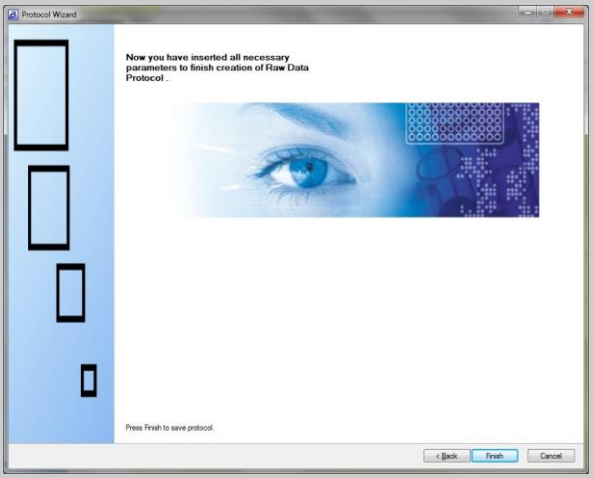
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

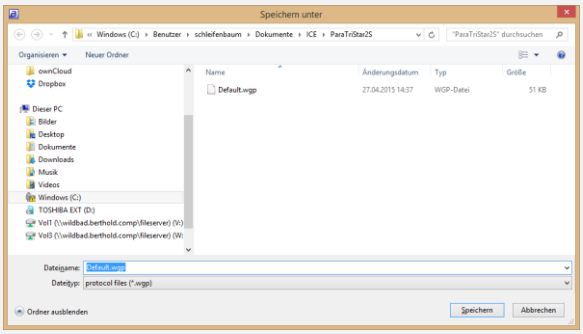
33. **Click <Next>**



34. **Click <Finish>**



35. Define the **protocol file name**
36. **Click <Save>**



7.6.2 Repeated measurement

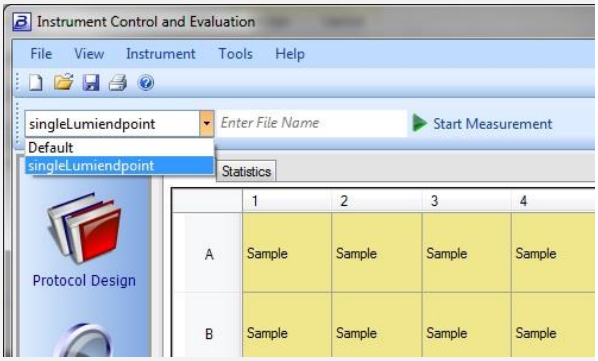
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

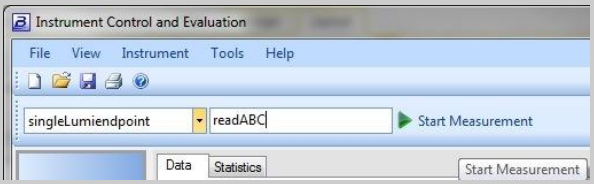
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

4. Insert the **microplate** with your samples:
well A1 facing the rear and left

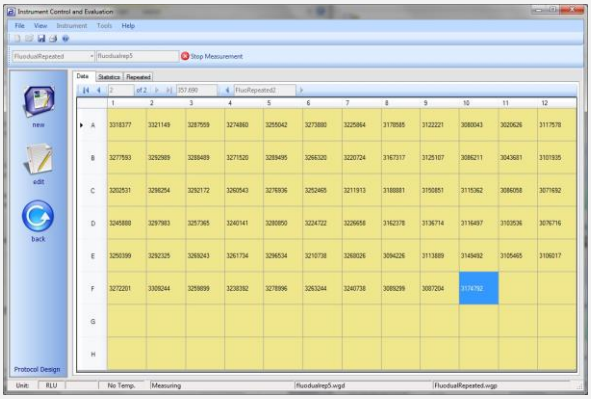
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

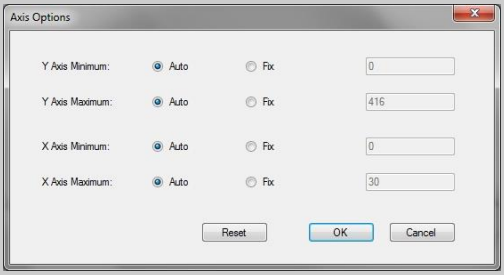
5. Click **<OK>**

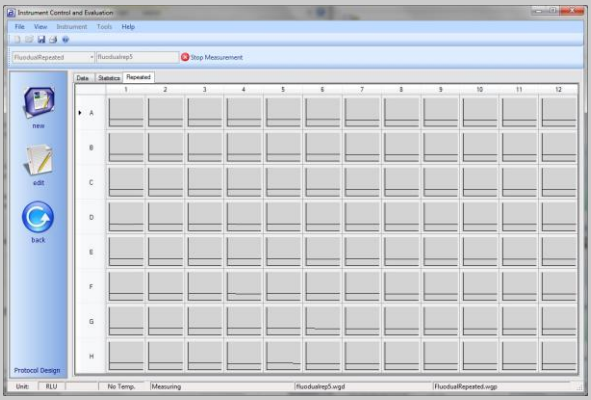
6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

   You may switch between the individual readings by clicking on the arrows

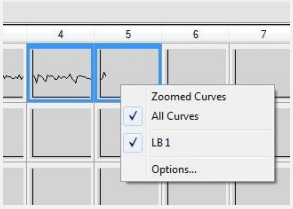


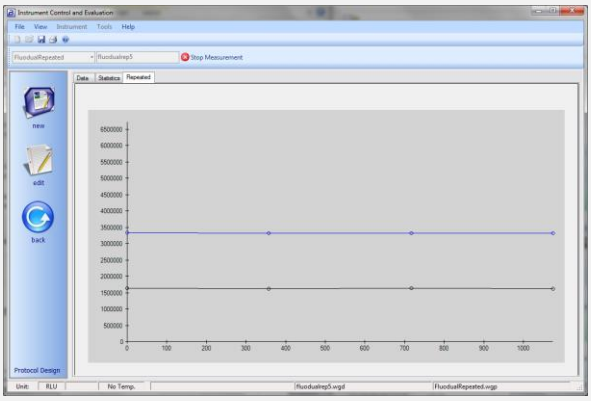
7. You also choose to view the curves by clicking the ***Repeated*** tab
8. The scale of the axes can be changed by right-clicking into the curves and selecting **Options…**



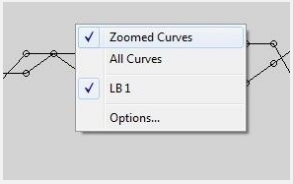


9. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Curves**

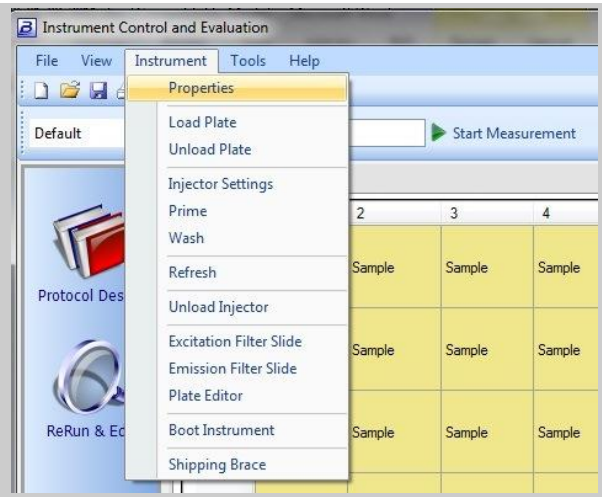




To un-zoom right-click into the zoomed view and select All Curves



10. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.7 Scanning Measurement

A scanning measurement mode is appropriate for assays with heterogeneous distribution of signal, e.g. cellular assays

7.7.1 Defining a protocol for a scanning measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

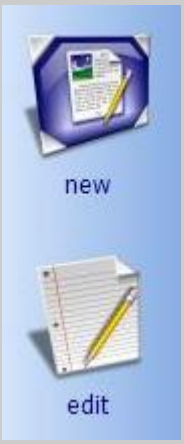
1. Click icon **Protocol Design** in the left-hand **Navigation** bar

   the navigation bar will appear in a new design

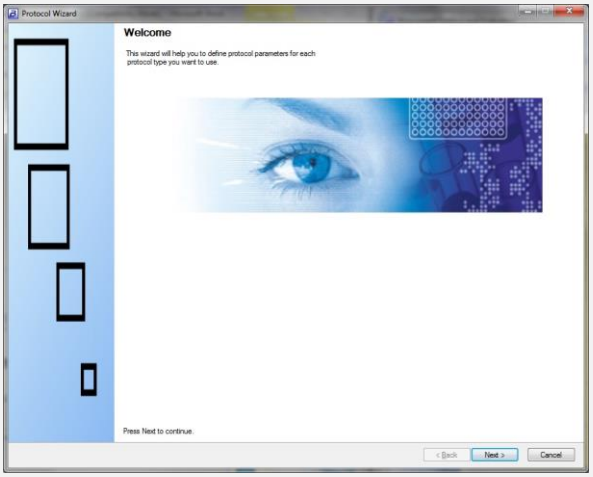
2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

   for editing an existing protocol use the **edit** icon

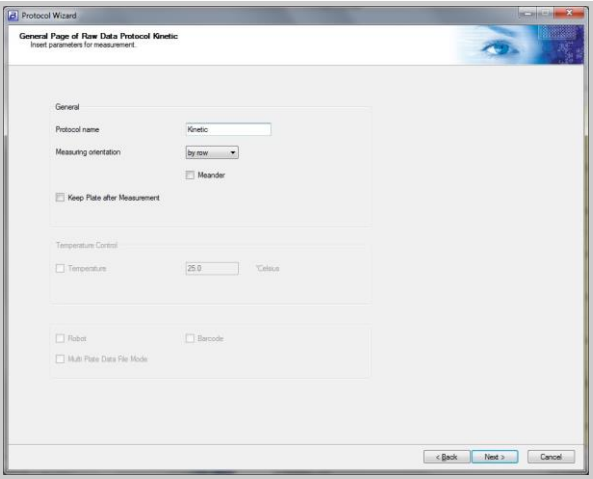


3. The start up screen of the protocol wizard will show up
   Click **<Next>**



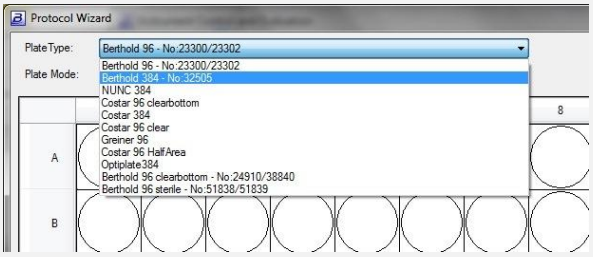
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



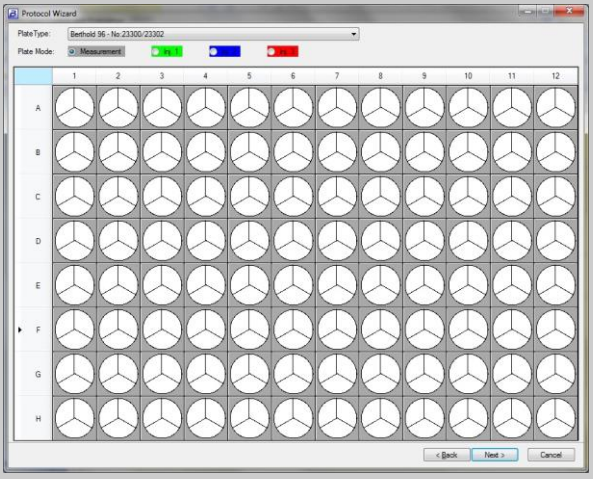
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

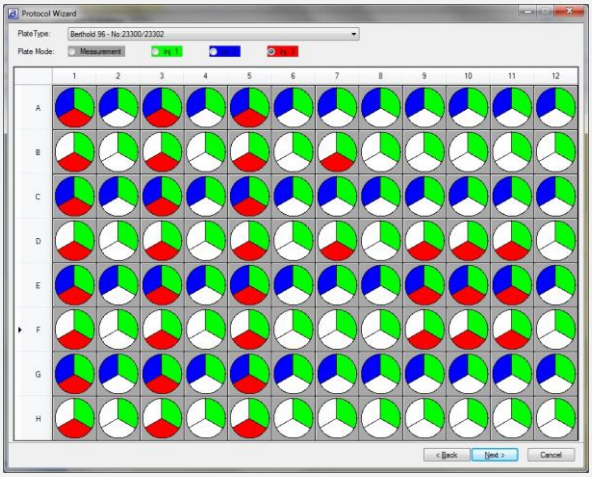
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

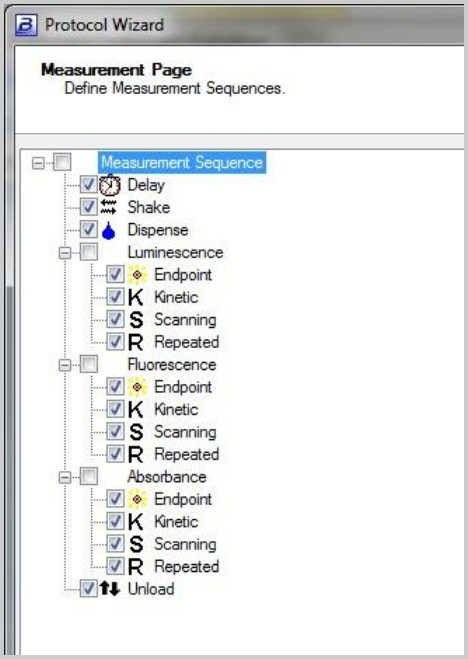
    Wells coloured in the respective colour are injected into

    Note: only wells to be measured can be injected into
14. Click **<Next>**



Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well

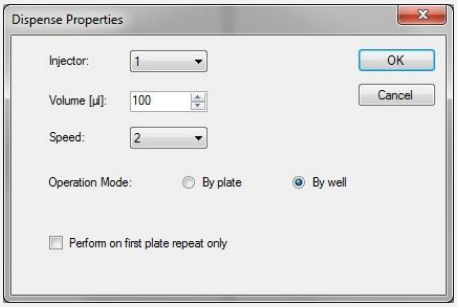


15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

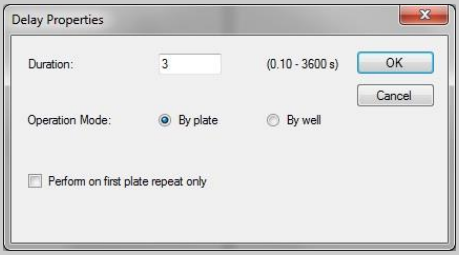
    In case additional reagent additions are required repeat this procedure for the other injector(s)



17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

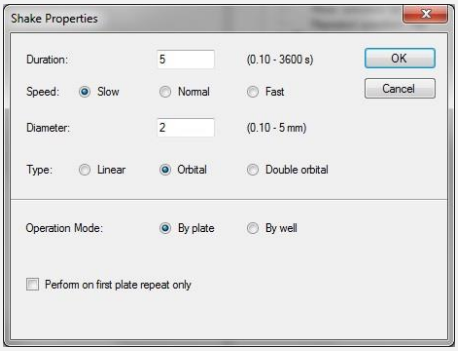
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Scanning*** in the Fluorescence section for a fluorescence scanning reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

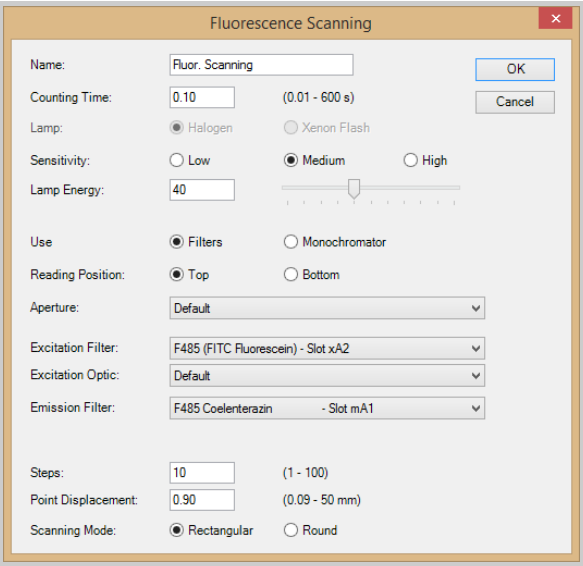
Reading Position	Choose reading from above (top) or below (Bottom) the plate.

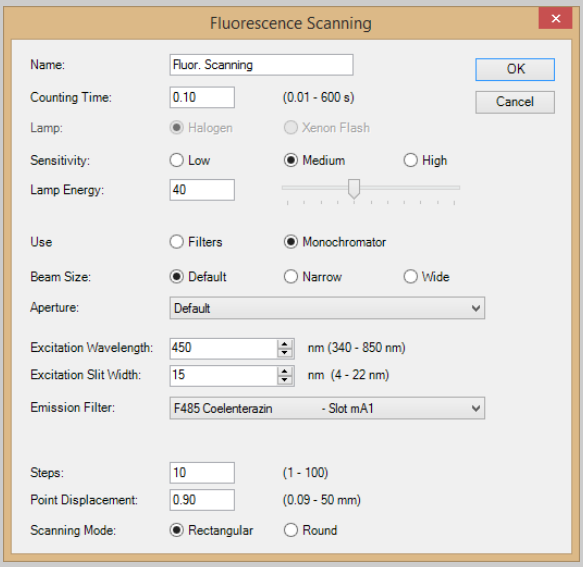
Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

22. Click **<OK>**





23. Double-click ***Scanning*** in the Absorbance section for a absorbance scanning reading

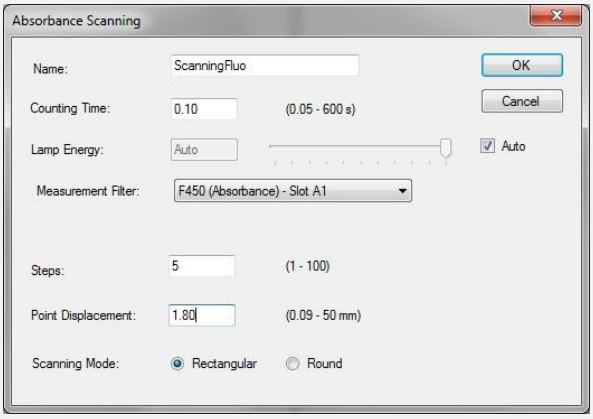
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator
Measurem. Filter	select from the list
Meas. Wavelength	set value
Meas. Slit Width	set value

Note: filters must be defined prior in the Instrument menu

Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

24. Click **<OK>**

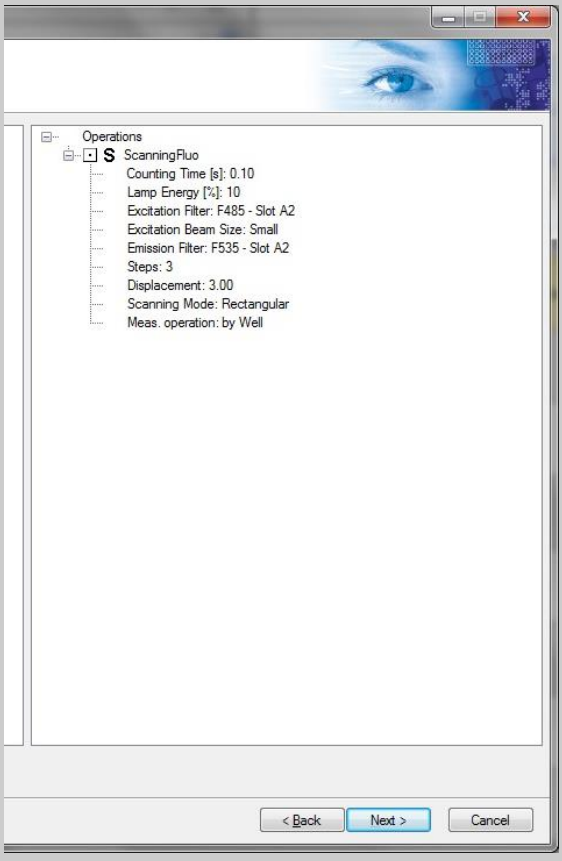


25. The **sequence of selected operations** will be displayed on the right-hand side

Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

26. Click **<Next>**



27. Define **Export** settings

    Type your **Header** specific for this protocol

    Select the data set by dragging from left to right

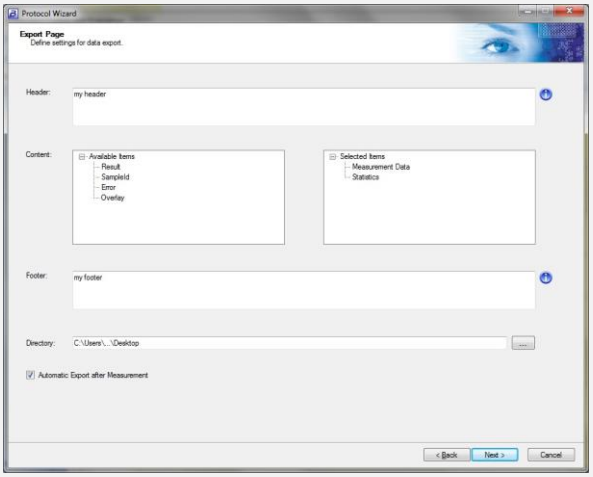
Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required

28. Click **<Next>**



29. Define **Print** settings

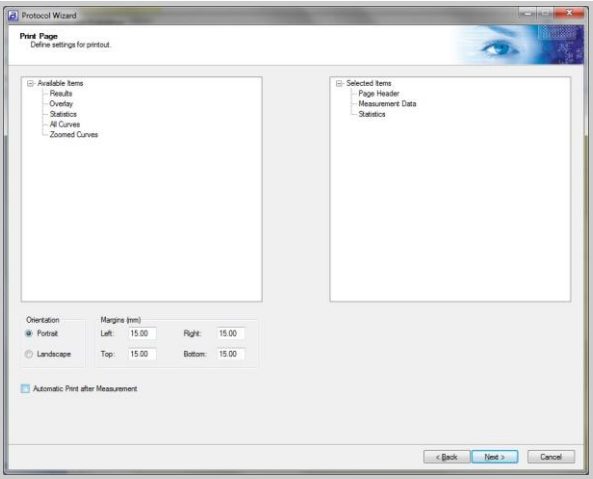
    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

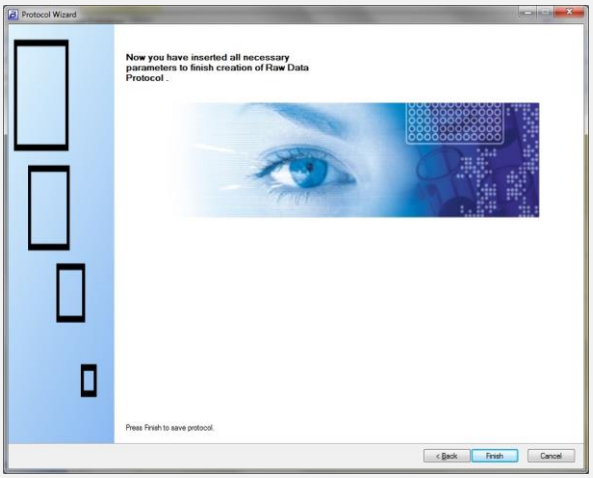
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

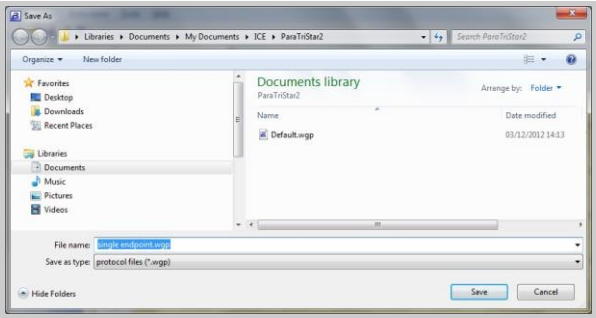
30. Click **<Next>**



31. Click **<Finish>**



32. Define the protocol **file name**
33. Click **<Save>**



7.7.2 Scanning measurement

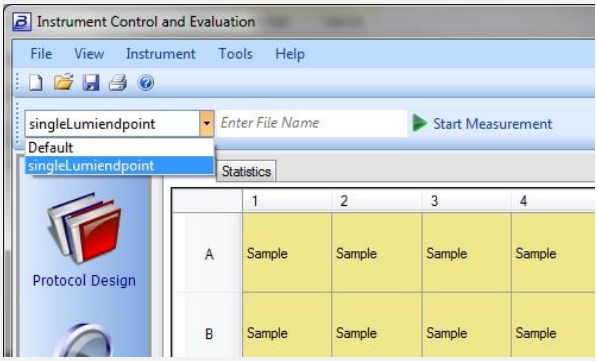
The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 8 of this manual.

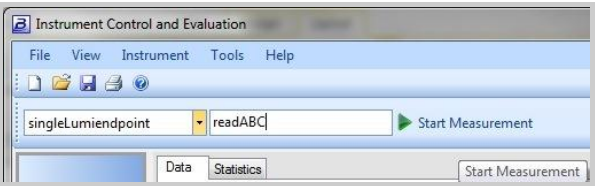
Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used



2. Enter a **file name** under which the measurement is to be stored



3. Click **<Start Measurement>**

4. Insert the **microplate** with your samples:
well A1 facing the rear and left

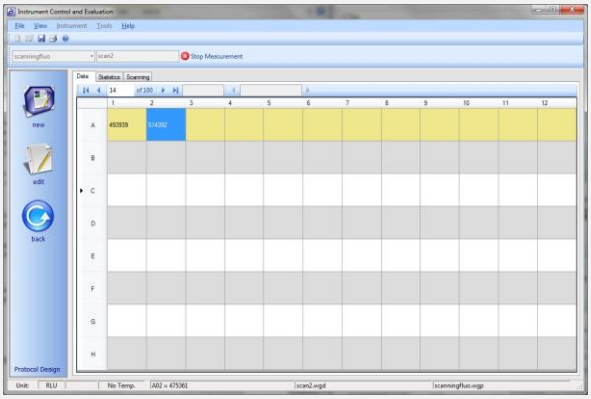
   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

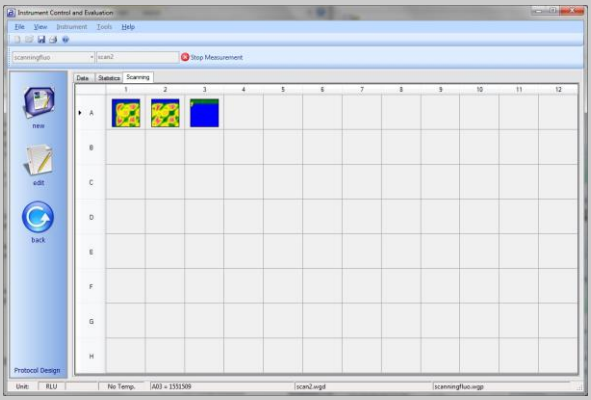
5. Click **<OK>**

6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

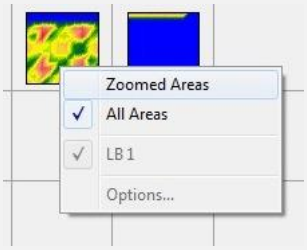
   You may switch between the individual readings by clicking on the arrows

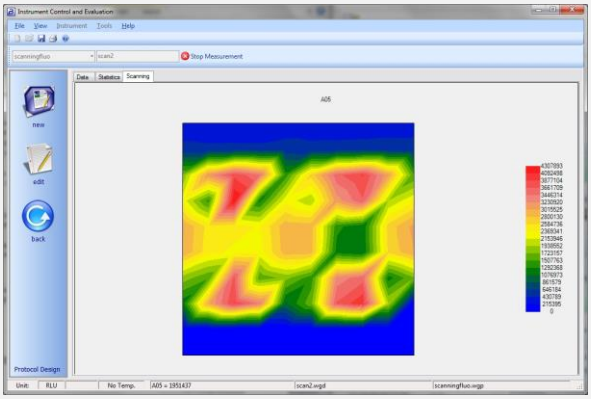


7. You also choose to view a graphical display by clicking the ***Scanning*** tab

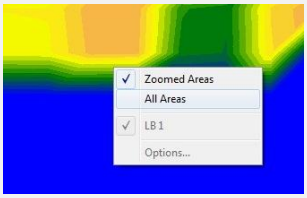


8. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Areas**

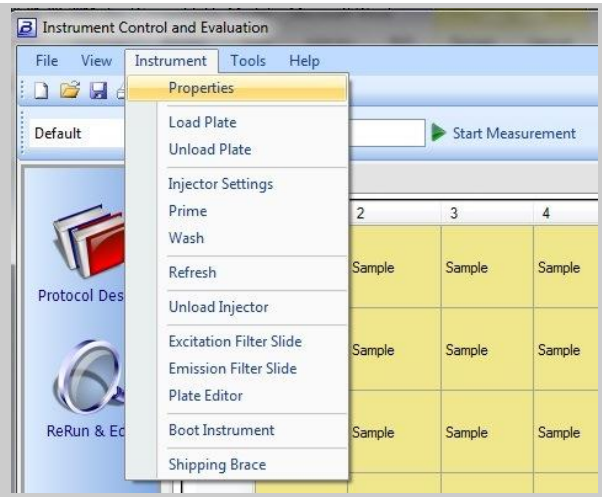




   To un-zoom right-click into the zoomed view and select All Curves



9. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



7.8 Wavelength Scanning Measurement

A wavelength scanning measurement mode is appropriate when the peak wavelengths of the fluorophores or dyes are unkown or when changes of the said are expected to change due to assay conditions, e.g. pH, polarity, enzymatic activities.

The TriStar² S is equipped with a monochromator in the excitation optics, thus absorbance scans or fluorescence excitation scans can be performed.

7.8.1 Defining a protocol for a wavelength scanning measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

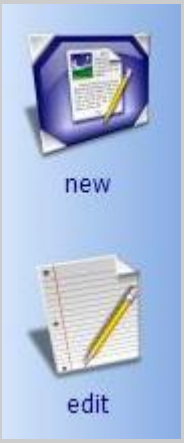
1. Click icon **Protocol Design** in the left-hand **Navigation** bar

   the navigation bar will appear in a new design

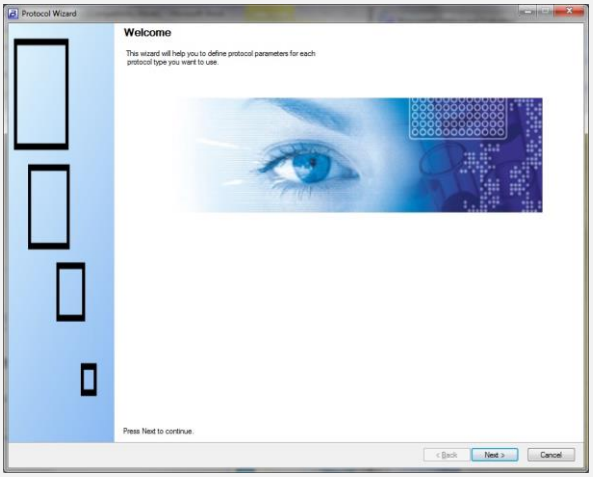
2. Click the **new** icon in the left-hand **Navigation** bar

   again, the navigation bar will appear in a new design

   for editing an existing protocol use the **edit** icon

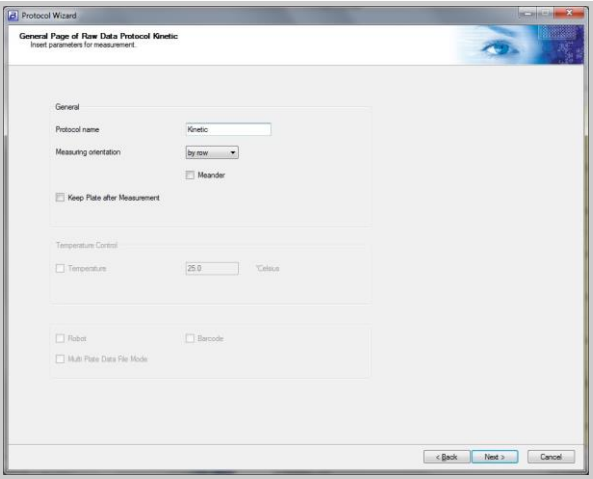


3. The start up screen of the protocol wizard will show up
   Click **<Next>**



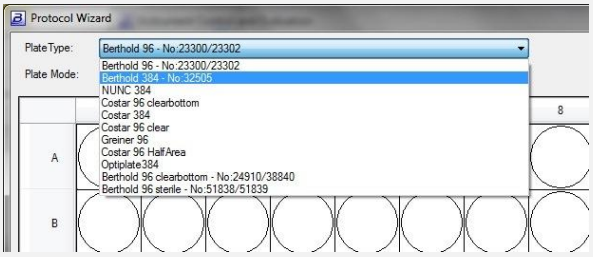
4. Enter a (descriptive) **Name** for your protocol
5. Define the **reading orientation**:
   by column or by row
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
8. Check **Temperature** to activate the temperature control for this protocol
9. Define the **target temperature**
   the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

   Robot, Barcode and Multi Plate Data File Mode are currently not active
10. Click **<Next>**



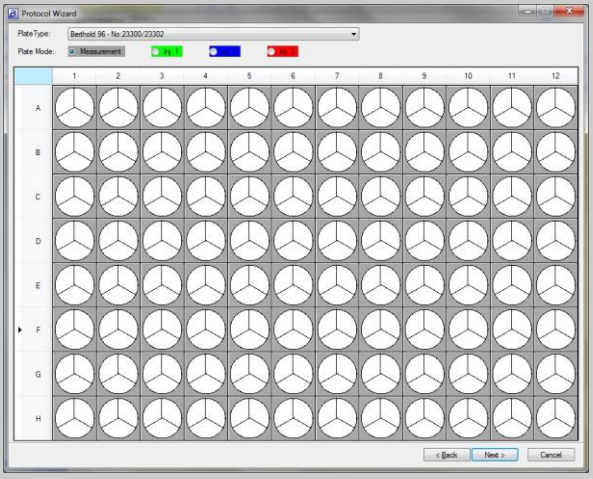
11. Select the **Plate Type** (microplate format)

    Note: the microplate has to be defined in the Plate Editor prior to defining a protocol



12. Select the wells to be measured by clicking the Measurement radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

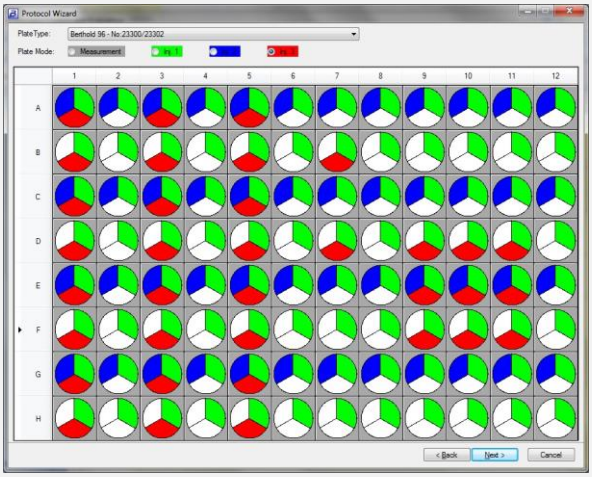
    Wells with a gray outside area are selected for measurement



13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
    - for the whole plate, click the top left corner
    - for a row, click the respective character
    - for a column, click the respective number
    - for an area, click and drag the mouse
    - for an individual well, click into it

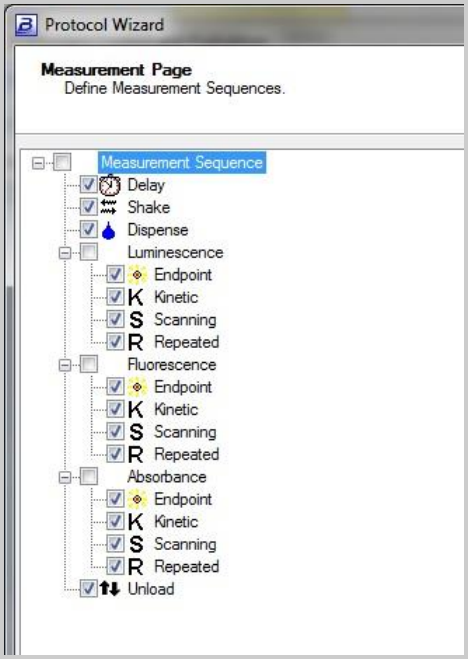
    Wells coloured in the respective colour are injected into

    Note: only wells to be measured can be injected into
14. Click **<Next>**



Define the Measurement Operations

- Available operations are shown on the left-hand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed
  by plate the operation will be executed for all selected before the consecutive operation is started
  by well all consecutive by well operations will be executed for a well before moving on to the next well

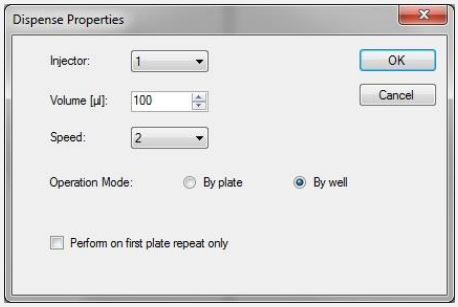


15. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

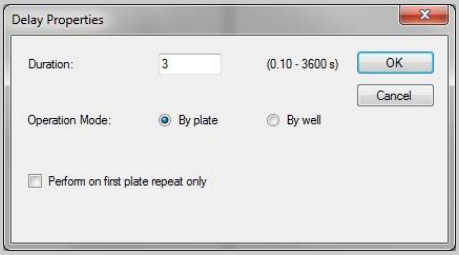
    In case additional reagent additions are required repeat this procedure for the other injector(s)



17. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

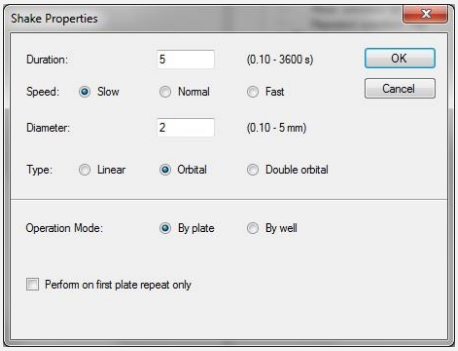
18. Click **<OK>**



19. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

20. Click **<OK>**



21. Double-click ***Spectral Scanning*** in the Fluorescence section for a fluorescence excitation wavelength scanning measurement

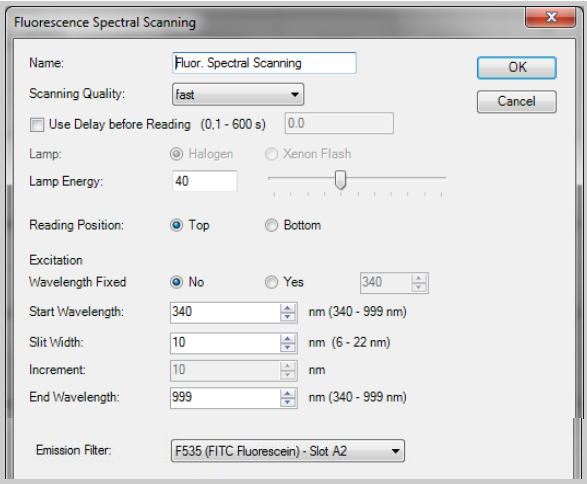
Name	give a (descriptive) name
Scanning quality	**fast** (10 nm increment), **high** (1 nm increment) or **custom**
Reading Position	Choose reading from above (top) or below (Bot tom) the plate.

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Start Wavelength	200 – 1000 nm (max. emission is 650 nm)
Slit Width	4 – 22 nm
Increment	1 - 50 nm
End Wavelength	200 – 1000 nm (max. emission is 650 nm)
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

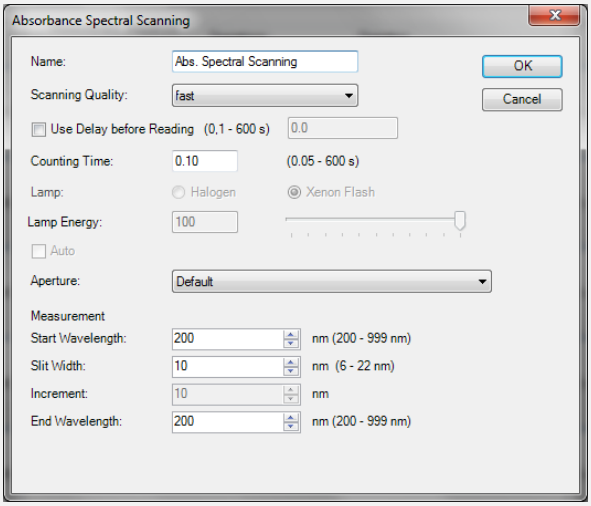
22. Click **<OK>**



21. Double-click ***Spectral Scanning*** in the Absorbance section for a absorbance scanning reading

Name	give a (descriptive) name
Scanning quality	**fast** (10 nm increment), **high** (1 nm increment) or **custom**
Start Wavelength	200 – 1000 nm
Slit Width	4 – 22 nm
Increment	1 - 50 nm
End Wavelength	200 – 1000 nm

22. Click **<OK>**

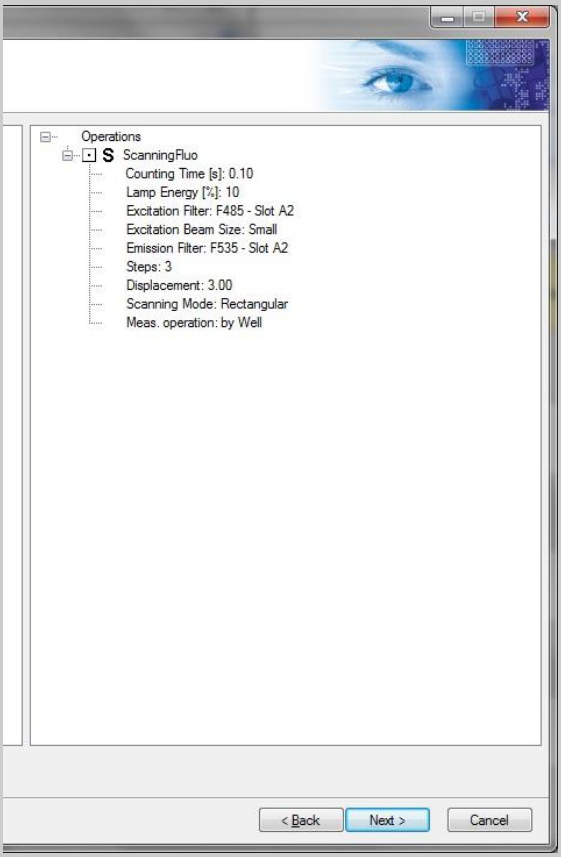


23. The **sequence of selected operations** will be displayed on the right-hand side

    Operations can be moved up or down by clicking on the operation and dragging them to the respective position

    Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

24. Click **<Next>**



25. Define **Export** settings

    Type your **Header** specific for this protocol

    Select the data set by dragging from left to right

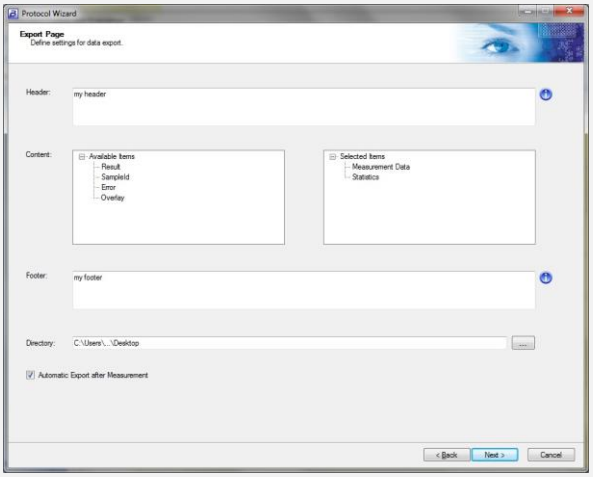
Sample ID	sample information
Measurement Data	readings
Result	calculated data
Error	any error codes
Overlay	well information
Statistics	measurement settings

    Type your **Footer** specific for this protocol

    Define **Directory** for the export file

    Check if **Automatic Export** is required

26. Click **<Next>**



27. Define **Print** settings

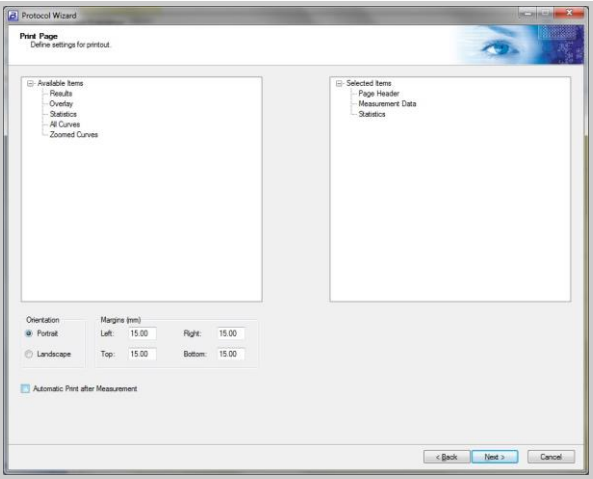
    Select the data set by dragging from left to right

Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

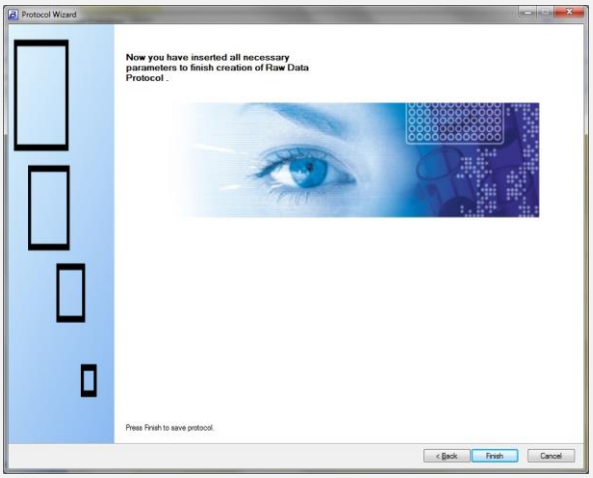
    Define **page orientation** and **margins**

    Check if **Automatic Print-out** is required

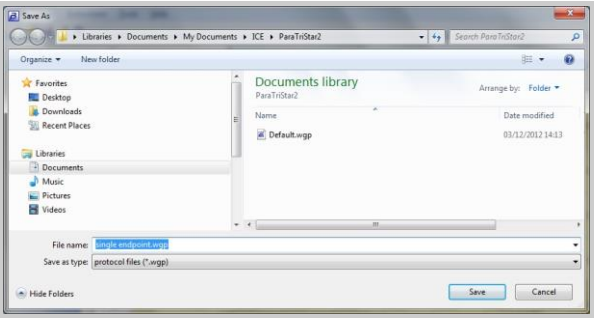
28. Click **<Next>**



29. Click **<Finish>**



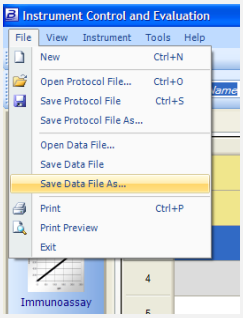
30. Define the protocol **file name**
31. Click **<Save>**



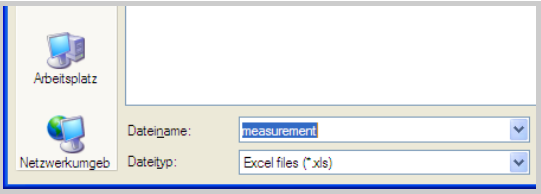
7.9 Data export and print-out

7.9.1 Direct data export

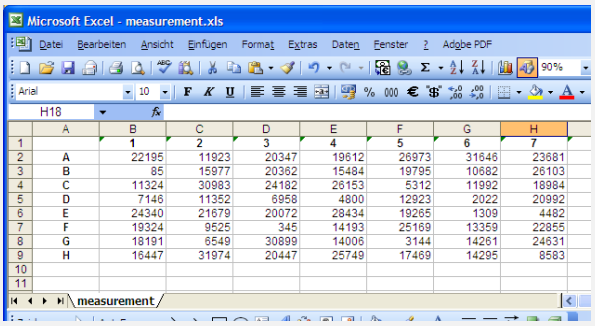
1. Click on **Save Data File As…** in the File menu



2. Select the file format, e.g. **Excel files (*.xls)**
3. Define the file name **without extension**
4. Select the appropriate folder
5. Click **<Save>**



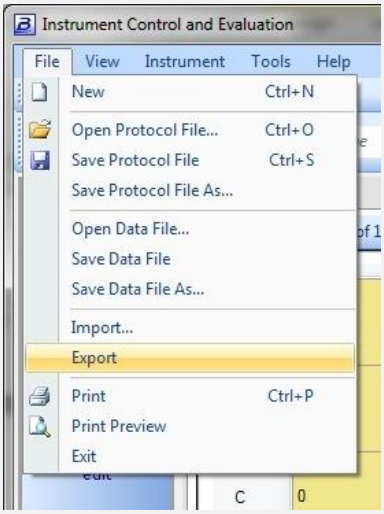
6. Open the *.xls file



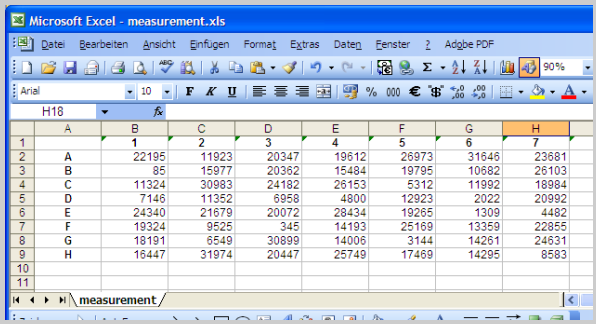
The export will be executed automatically if selected in the respective protocol file.

7.9.2 Data export via Export

1. Click on **Export** in the File menu
2. An EXCEL file will be created with file name resembling that of the data file



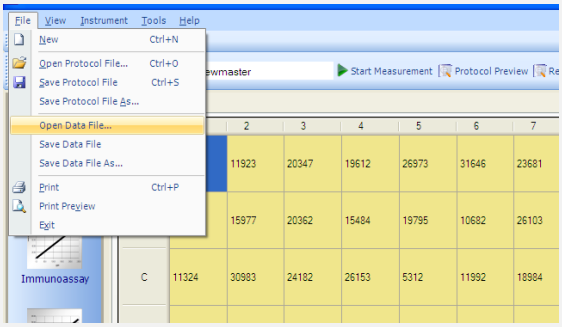
3. Open the *.xls file



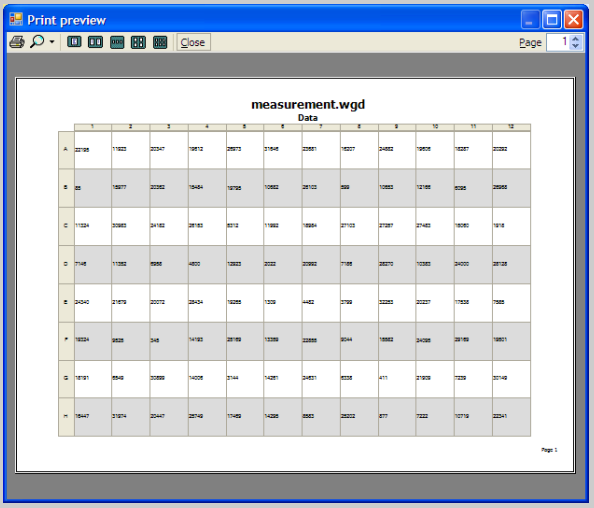
The export will be executed automatically if selected in the respective protocol file.

7.9.3 Direct data print-out

1. If not opened already open the respective data file by selecting **Open Data File** in the **File** menu



2. Select **Print Preview** in the **File** menu to get a preview of the print-out
3. Select **Print** in the **File** menu to start printing the data



The print-out will be executed automatically if selected in the respective protocol file.

8. Operation with Mikrowin 2010

Running measurements on the TriStar² S is straight forward. The procedure is the same for all types of assay types, e.g. Raw Data, Dual Label, Kinetic, Repeated and Scanning. A measurement can be carried out immediately after a stored protocol is selected. At the end of each measurement the results are stored and may be printed or exported.

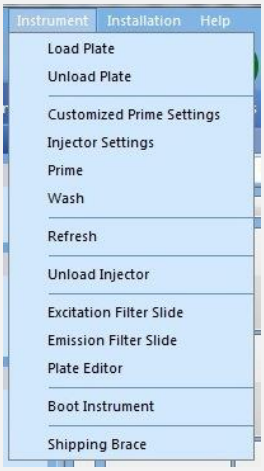
Result file names can be given without limitation. The extension is fixed, though. This is valid for measurement protocols as well.

8.1 Adding and Editing Microplate Dimensions

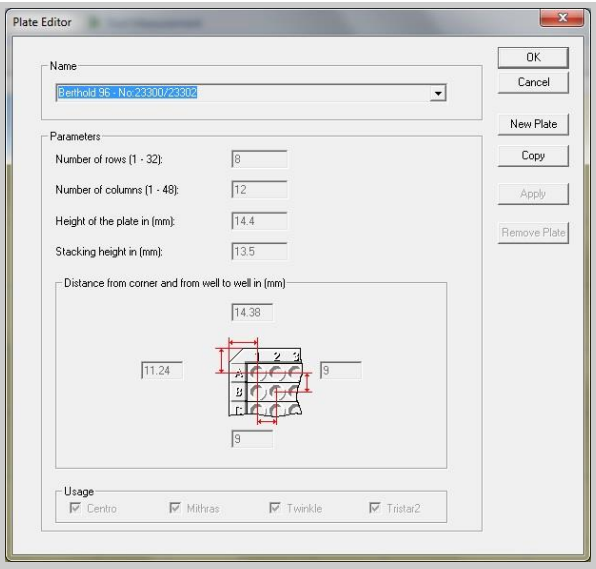
Microplates can differ in their dimensions dependent on brand and type. Please refer to the manufacturer´s most recent information for exact dimensions of the microplates.

Microplates must be defined in the plate editor prior to defining a measurement protocol.

1. Click **Plate Editor** in the **Instrument** menu



2. Click **<New Plate>** or select a plate with matching well format and click **<Copy>**



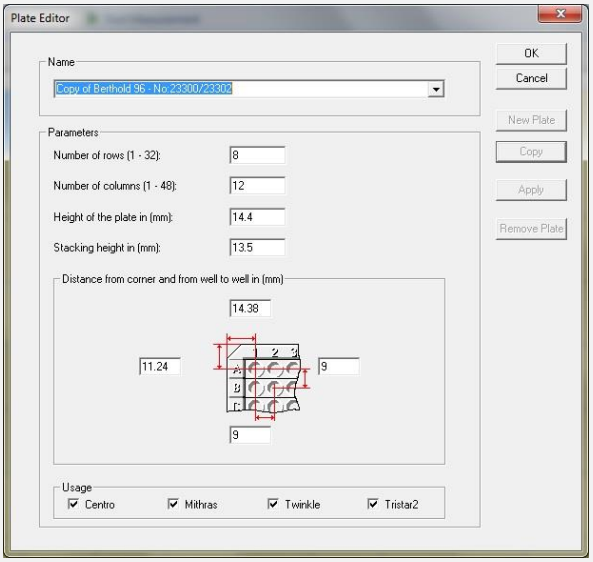
3. Assign a (descriptive) **Name**
4. Insert the **Number of rows**, e.g. ***8*** for a 96 well plate
5. Insert the Number of columns, e.g. ***12*** for a 96 well plate
6. Insert the total **Height** of the microplate

   most 96 and 384 well plates are between 14 and 15.5 mm
7. Insert the **Stacking height** of the microplate

   the stacking height is the resulting height (the visible part) when plates are put on top of each other (e.g. in a plate stacker)

   in case this information is not available from the plate manufacturer the stacking height can be derived by stacking 2 plates and measuring the total height; by subtracting the regular height of one of the plates the resulting value will be the stacking height
8. Insert the distance between the left outer edge of the plate and the center of well A1
9. Insert the distance between to upper outer edge of the plate and the center of well A1
10. Insert the distance between the well centers of consecutive rows (vertical well distance)
11. Insert the distance between the well centers of consecutive columns (horizontal well distance)
12. Check the usage ***TriStar2***

    you may check additional instruments in case you have multiple instruments in operation
13. Click **<Apply>**
14. Click **<OK>**



15. The plate can now be used in the protocol files

8.2 Single Raw Data measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine ATP content, single reporter gene expression, activities of caspases, kinases and many other enzymes.

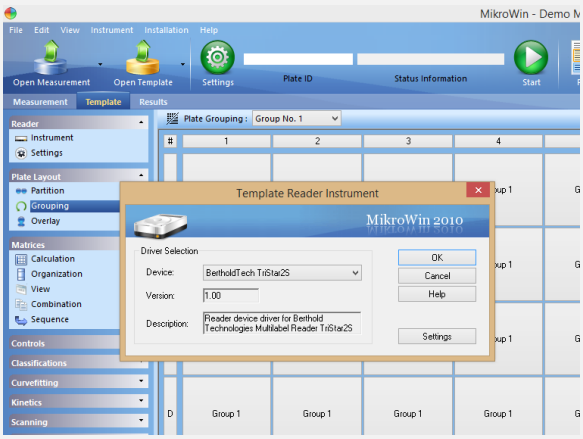
8.2.1 Defining a Single Endpoint protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click **Template** tab and then click **Instrument**
2. Select **BertholdTech TriStar2S**

   You can omit this step when only a single instrument is connected.

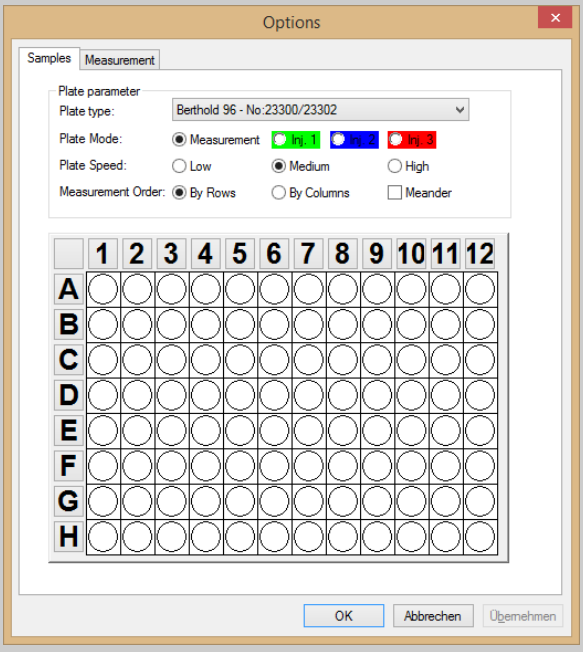
3. Click **<Settings>**



4. Select the **Plate Type** (microplate format)

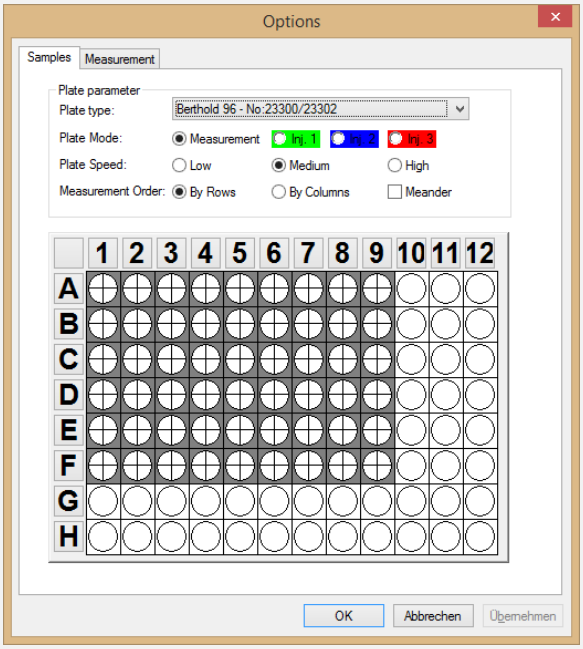
   Note: the microplate has to be defined in the Plate Editor prior to defining a protocol

5. Define the **reading orientation**:
   by columns or by rows
6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top



7. Select the wells to be measured by clicking the Measurement radio button
   - for the whole plate, click the top left corner
   - for a row, click the respective character
   - for a column, click the respective number
   - for an area, click and drag the mouse
   - for an individual well, click into it

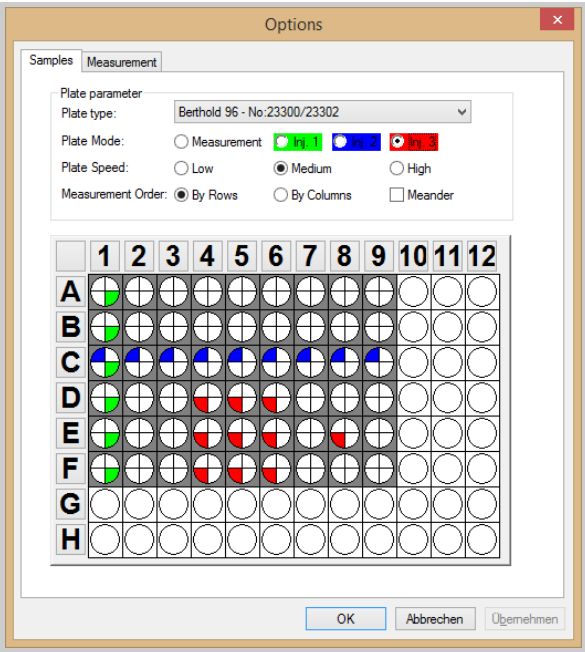
   Wells with a gray outside area are selected for measurement



8. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
   - for the whole plate, click the top left corner
   - for a row, click the respective character
   - for a column, click the respective number
   - for an area, click and drag the mouse
   - for an individual well, click into it

   Wells coloured in the respective colour are injected into

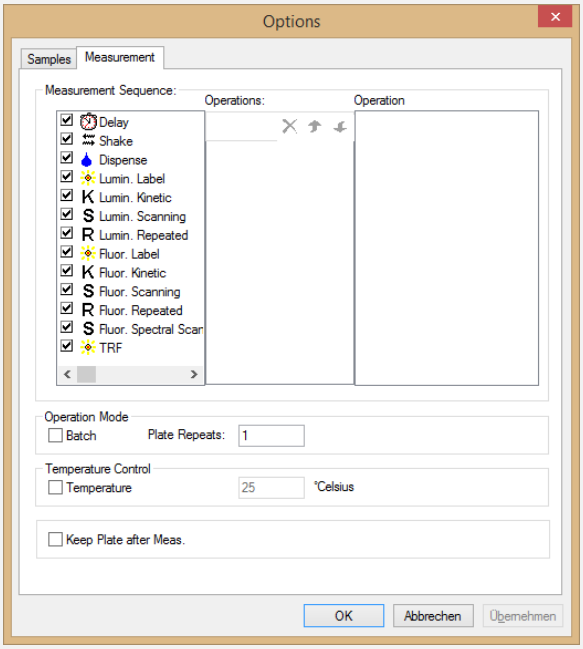
   Note: only wells to be measured can be injected into



9. lick onto the **Measurement** tab

   Define the Measurement Operations

   - Available operations are shown on the left-hand area
   - Allowed operations are indicated by a check mark
   - Double-clicking an operation open the specific properties dialogue
   - Confirming the settings by clicking **<OK>** selects the operation and adds it to the operation sequence visible in the right-hand area
   - The operation may be executed
     by plate the operation will be executed for all selected before the consecutive operation is started
     by well all consecutive by well operations will be executed for a well before moving on to the next well

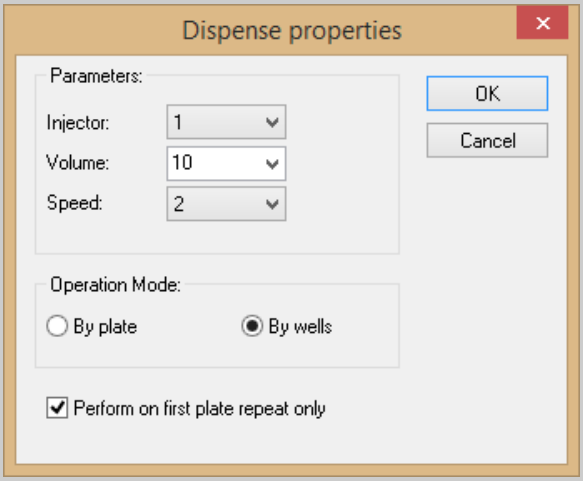


10. Double-click ***Dispense*** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

11. Click **<OK>**

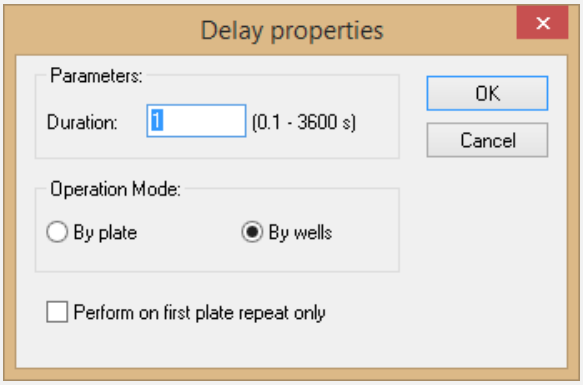
    In case additional reagent additions are required repeat this procedure for the other injector(s)



12. Double-click ***Delay*** in case an delay/incubation time is required

Duration	0.1 to 3600 s
Operation Mode	by plate or by well

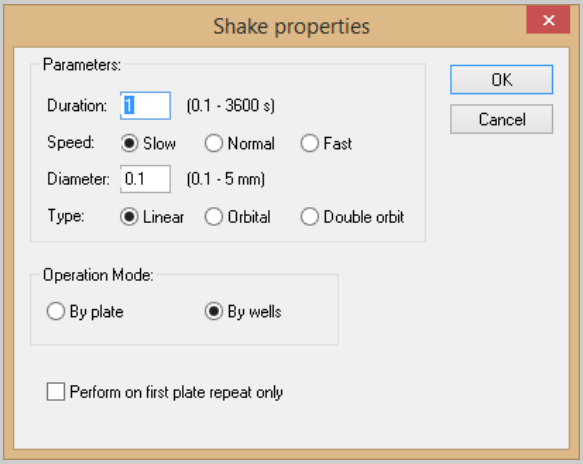
13. Click **<OK>**



14. Double-click ***Shake*** in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Type	linear, orbital, double-orb.
Operation Mode	by plate or by well

15. Click **<OK>**



16. Double-click ***Lumin.Label*** for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter

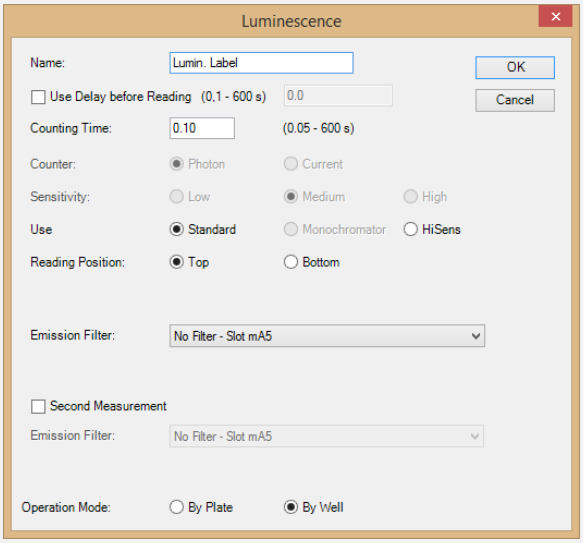
Note: filters must be defined prior in the Instrument menu

Reading Position	Choose reading from above (top) or below (Bottom) the plate

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Operation Mode	by plate or by well

17. Click **<OK>**



18. Double-click ***Fluor. Label*** in the Fluorescence section for a fluorescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

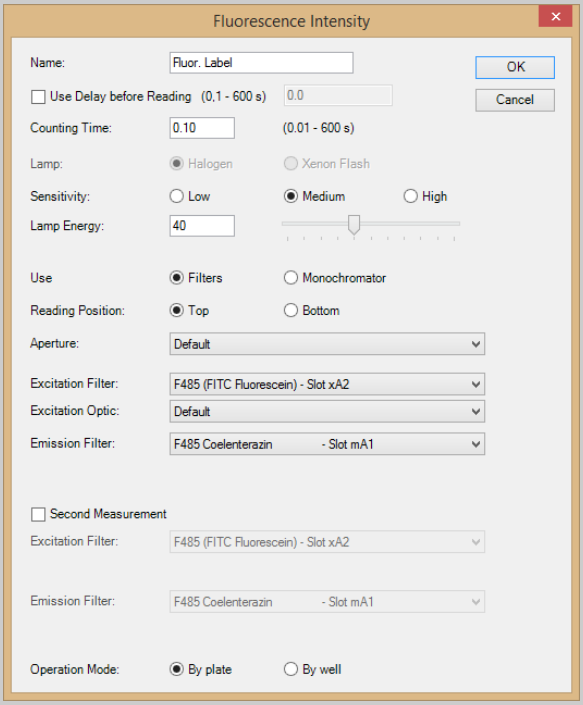
Note: filters must be defined prior in the Instrument menu

Reading Position	Choose reading from above (top) or below (Bottom) the plate

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Operation Mode	by plate or by well

19. Click **<OK>**



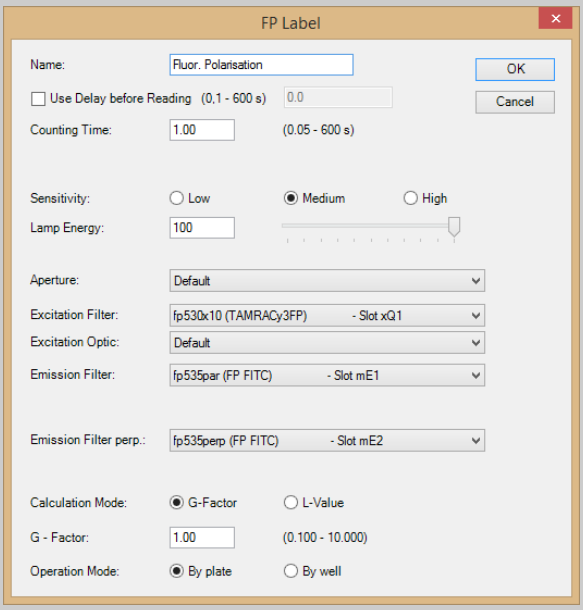
20. Double-click ***FP Label***

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Excitation Filter	choose the appropriate excitation filter
Emission Filter	choose the appropriate emission filter for vertically oriented fluorescence
Emission Filter perp.	choose the appropriate emission filter for perpendicularly oriented fluorescence

Note: FP filters use precisely aligned polarisation filters. For ideal results only use special FP fiter slides (

Note: filters must be defined prior in the Instrument menu

G-Factor	Enter the correct G factor for your assay and this instrument derived from a G factor determination measurement.
L-Value	Enter the correct L value for your assay and this instrument derived from a L value determination meas



urement.

Operation Mode	by plate or by well

21. Click **<OK>**

22. Double-click ***Absorbance*** for an absorbance reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator
Measurem. Filter	select from the list
Meas. Wavelength	set value
Meas. Slit Width	set value

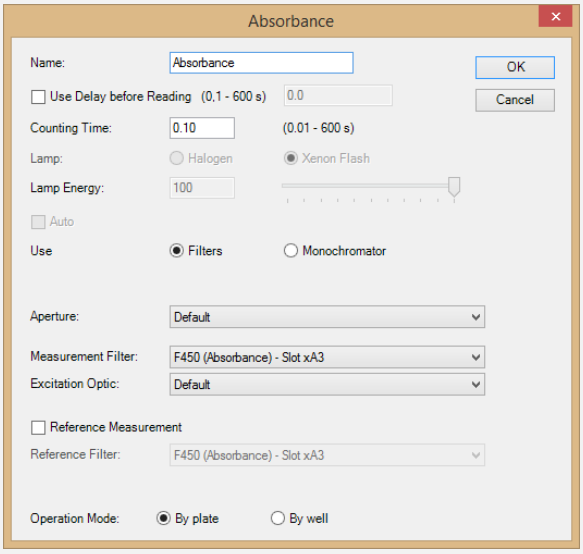
Note: filters must be defined prior in the Instrument menu

Reference Measurement

Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter	select from the list
Operation Mode	by plate or by well

23. Click **<OK>**



24. The **sequence of selected operations** will be displayed in the center column

Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

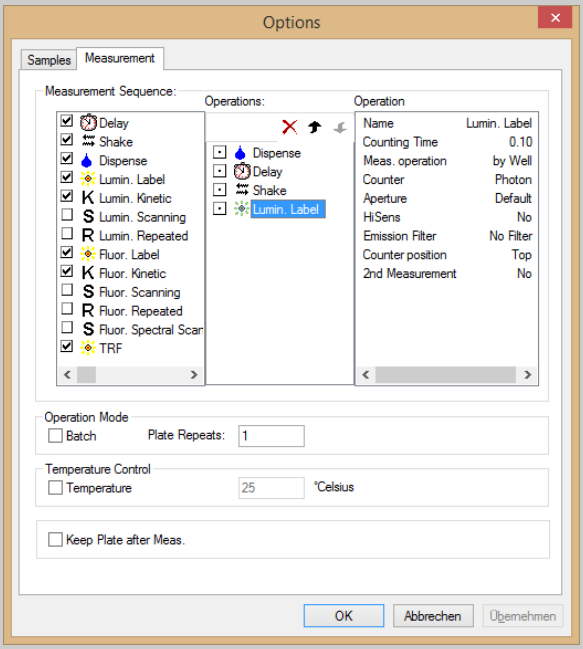
Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

25. Check **Batch** and define the number of plates in **Plate Repeats** in case you want a number of plates to be stored into a single data file

Note: this setting can only be used in single endpoint measurements

26. Define a number in **Plate Repeats** only in case you want the selected operations to be repeatedly executed

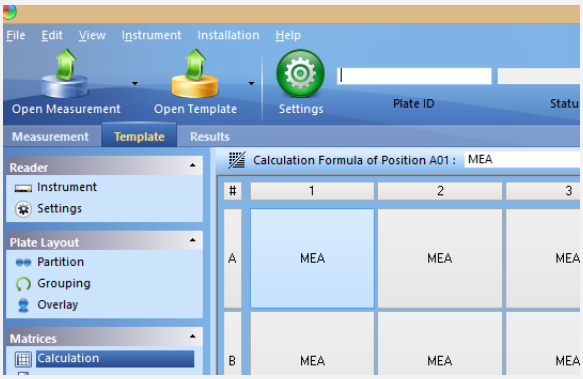


27. Check **Temperature** to activate the temperature control for this protocol
28. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active
29. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
30. Click **<OK>**
31. Click **<OK>** once more
32. By default the plain measurement data will be located on **Result matrix 2 "Reader_Values"**

    For export und print you have to refer to this matrix.

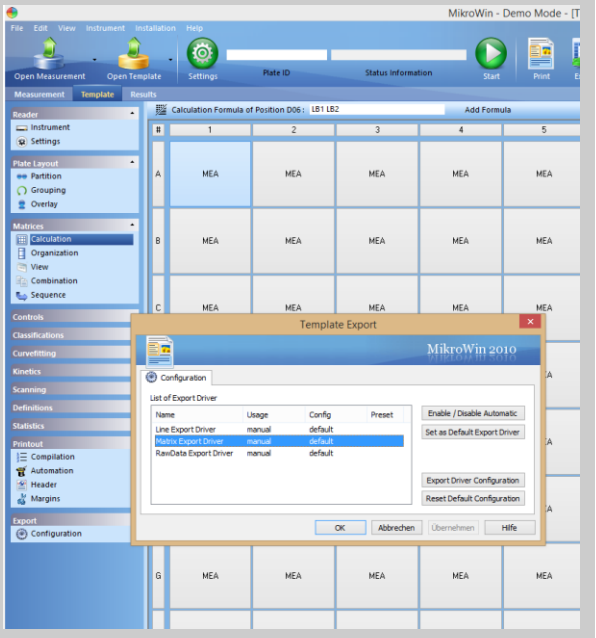
    Should you wish to define any additional calculations please refer to the Mikrowin manual.



33. To activate automatic export choose the **Template** tab and click **Configuration** in the **Export** section in navigation panel.
34. Select the appropriate export driver

Line	table/list format
Matrix	microplate layout
RawData	all measurement values

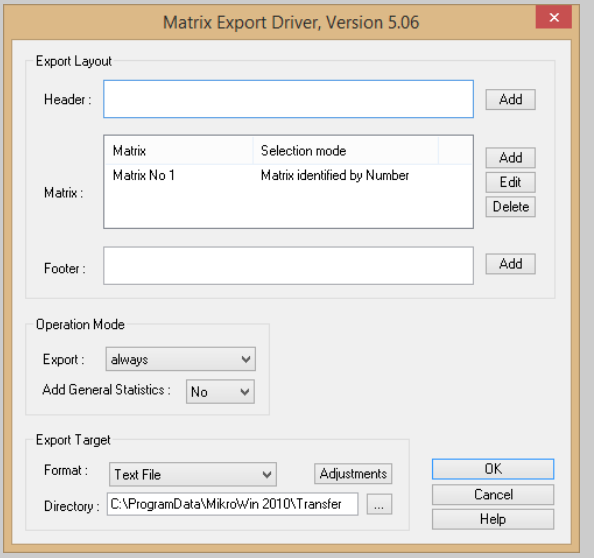
    The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.
35. Click **Enable/Disable Automatic**
36. You may click **Export Driver Configuration** should you require any changes of ***data selec-***



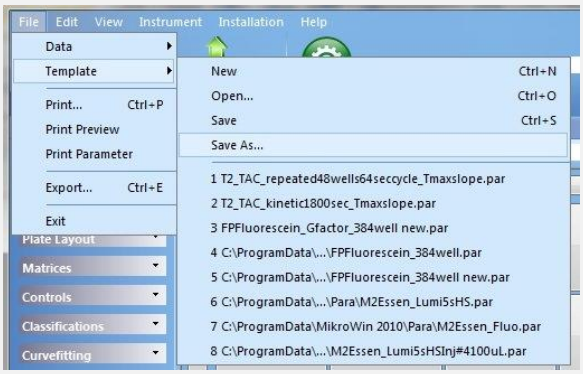
tion, ***file format*** or ***location***

37. Click **<OK>**

38. Click **<OK>**



39. Go to **File | Template | Save As…**
40. Create and/or select an appropriate directory, e.g. ***ParaTriStar2***
41. Select the file type **Mikrowin Para File (*.par)**
42. Type a meaningful **file name**
43. Click **<Save>**



8.2.2 Measurement with a Single Endpoint protocol

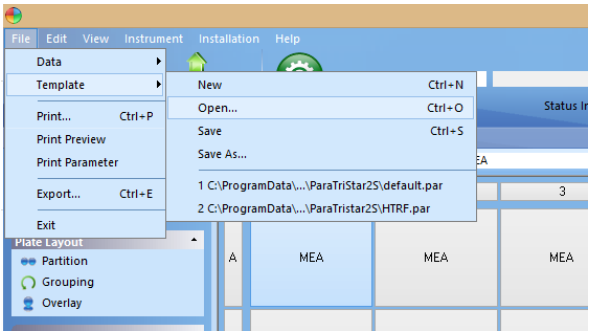
The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

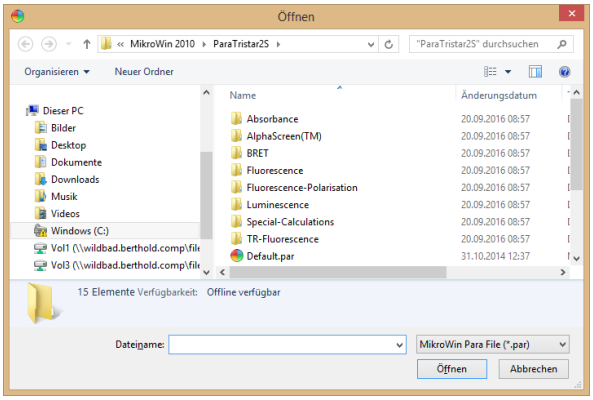
Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

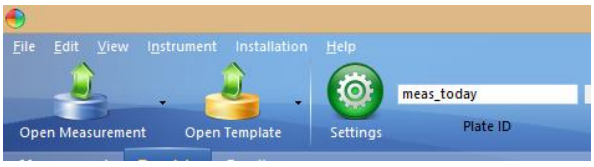
Note: Make sure the appropriate plate frame is inserted

1. Click **Open** in the **File/Template** menu
2. Select **File of type**: Mikrowin Para File
3. Select the appropriate file
4. Click **<Open>**





5. Enter a **file name** under which the measurement is to be stored



6. Click **<Start>**

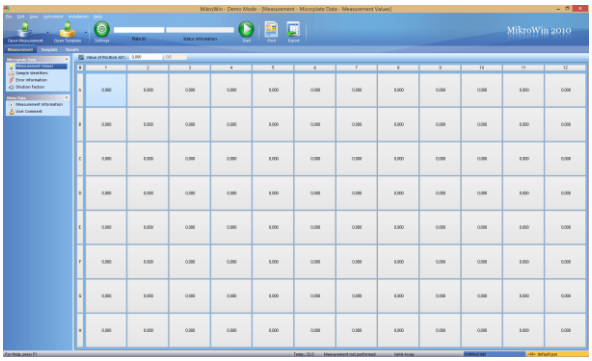
7. Insert the **microplate** with your samples:
   well A1 facing the rear and left

   Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

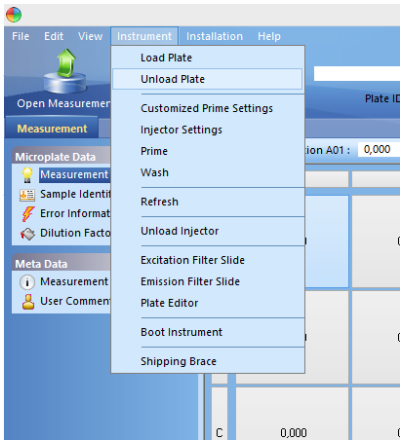
   Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates and for bottom reading protocols.

8. Click **<OK>**

9. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed



10. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument



8.3 Dual Label Assay measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine dual reporter gene expression.

8.3.1 Defining a Dual Label protocol

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Lumin.Label*** for a luminescence reading

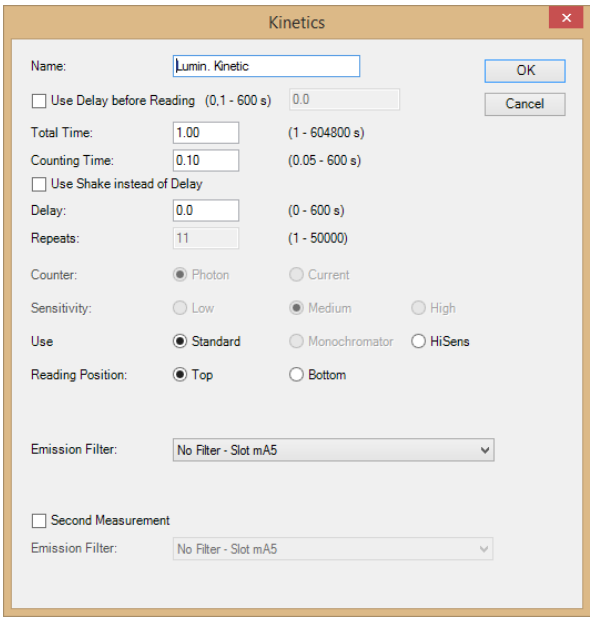
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Reading Position	above (top) or below (Bottom) the plate. Usually: Top

    Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Emission Filter	usually: No Filter

    Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well



17. Double-click ***Lumin.Label*** once more and define the settings for the second reading

    For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

    You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

18. Click **<OK>**

19. Double-click ***Fluor. Label*** for a fluorescence reading

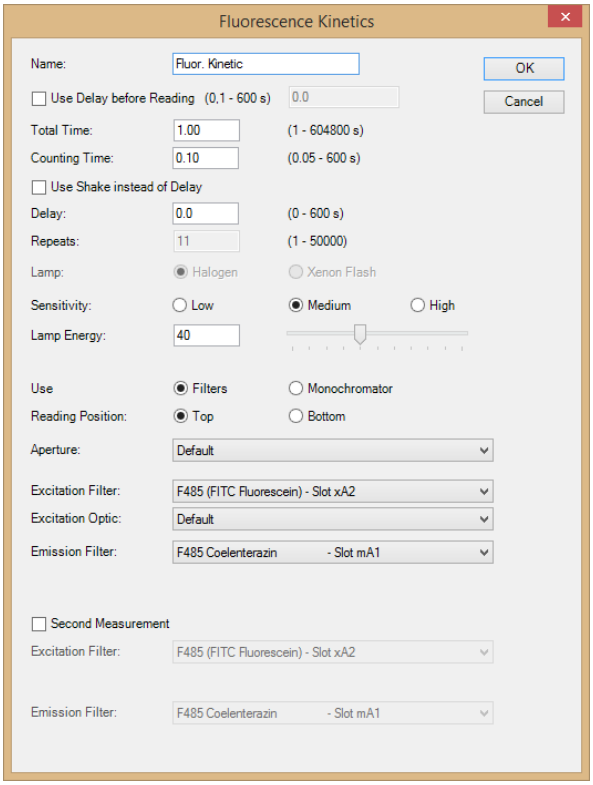
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Reading Position	above (top) or below (Bot tom) the plate. Usually: Top

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Use	Filters or Monochromator
Excitation Filter	select from the list
Emission Filter	select from the list
Meas. Wavelength	set value
Meas. Slit Width	set value

Note: filters must be defined prior in the Instrument menu

Operation Mode	by plate or by well



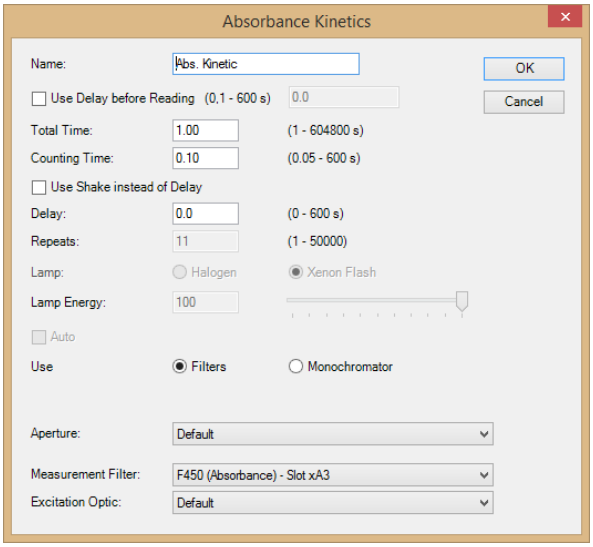
20. Double-click ***Fluor.Label*** once more and define the settings for the second reading

    For very fast switching between the first and the second reading, e.g. for fast BRET kinetics or for ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

    You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

21. Click **<OK>**

22. Double-click ***Absorbance*** for an absorbance reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Use	Filters or Monochromator
Measurem. Filter	select from the list
Meas. Wavelength	set value
Meas. Slit Width	set value

Note: filters must be defined prior in the Instrument menu

Reference Measurement

Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter	select from the list
Operation Mode	by plate or by well

23. Double-click ***Absorbance*** once more and define the settings for the second reading

    For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

    You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

24. Click **<OK>**

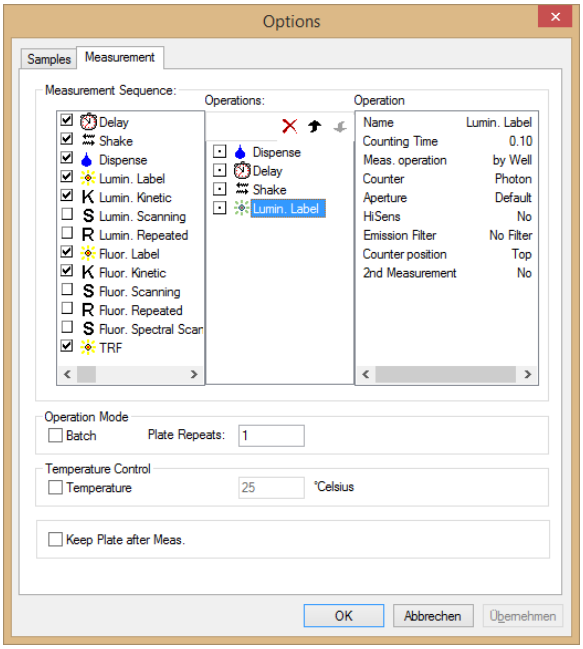
25. The **sequence of selected operations** will be displayed in the center column

    Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

    Operations can be deleted by highlighting and clicking the cross

    Details of the operation highlighted can be viewed on the right column

    An operation can be edited by double-clicking on it in the center column



26. Do <u>not</u> check **Batch**

    Note: this setting can only be used in single endpoint measurements

27. Define a number in **Plate Repeats** only in case you want the selected operations to be repeatedly executed

28. Check **Temperature** to activate the temperature control for this protocol
29. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active

30. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

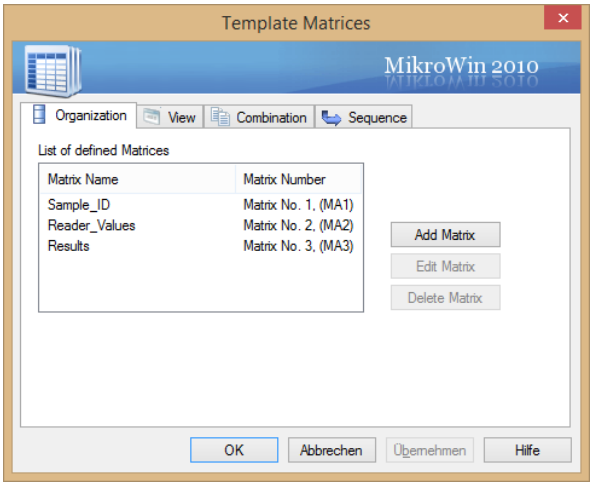
31. Click **<OK>**

32. Click **<OK>** once more

For further calculations of the measurements follow the next steps:

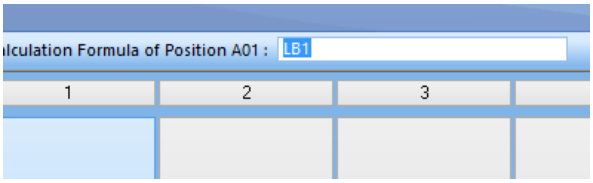
33. Select the **Template** tab and click **<Organization>** in the Matrices section. Click **<Add Matrix>**
34. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **first reading**

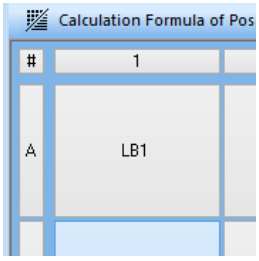
    Do the same for Matrix No. 3 (rename to e.g. **second reading**) and Matrix No. 4 (rename to e.g. **ratio**)
35. Click **<OK>**



For export und print you have to refer to this matrices.

36. Click on **Calculation** in the **Matrices** section
37. Click on the 2 **first reading** tab
38. Type **LB1** into the Calculation Formula: LB1 = Label 1 = first of readings
39. Click the double-cross to assign for all wells





40. Proceed with the two other matrices alike:

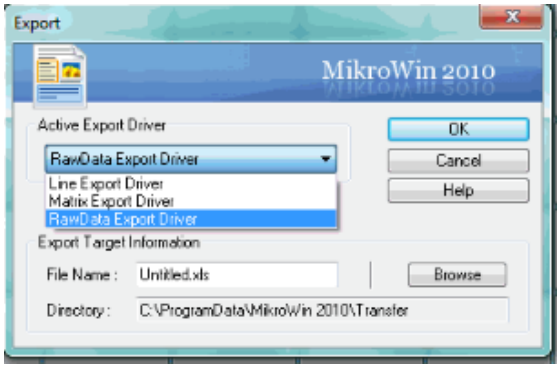
    3 **second reading LB2**
    LB 2 = Label 2 = second of readings

    4 **ratio MA2/MA3**
    MA2 = Matrix 2, MA3 = Matrix 3, MA2/MA3 = values of Matrix 2 divided by values of Matrix 3

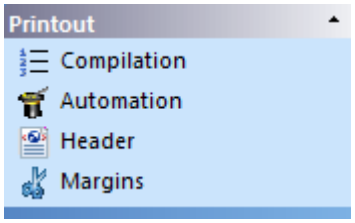
Should you wish to define any additional calculations please refer to the Mikrowin manual.

41. To activate automatic export click **Export Set-up…** in the **File** menu
42. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.



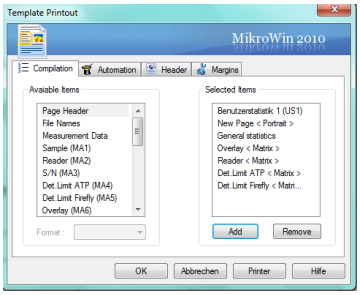
43. Click **<OK>**



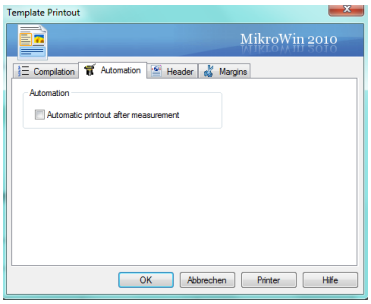
44. Open the **Printout** section in the **Template** tab
45. Click **Compilation** to enter the following data

Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
first	measured data (matrix 2)*
second	measured data (matrix 3)*
ratio	ratio of readings (matrix 3)*
Gen. Statistics	measurement settings

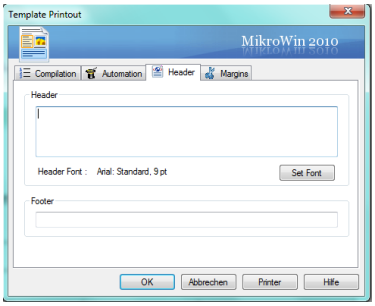
* the selection and content depends on the matrix definition done in the Calculation section



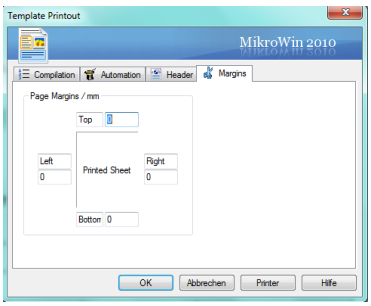
46. Check if **Automatic Print-out** is required



47. Type a **header** and/or **footer**

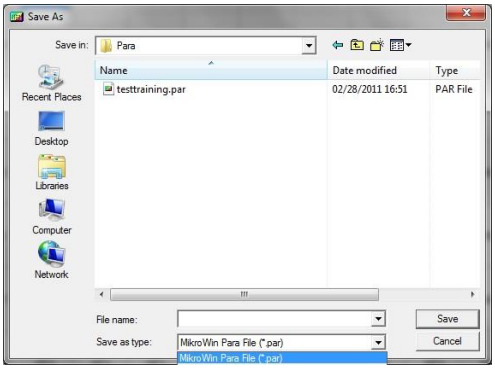


48. Select the page **margins** for the printout



49. Click **<OK>**

50. Go to **File | Para | Save As…**
51. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2S***
52. Select the file type **Mikrowin Para File (*.par)**
53. Type a meaningful **file name**
54. Click **<Save>**



8.3.2 Measurement with a Dual Label Assay protocol

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2 "Measurement with a single endpoint rotocol"**.

8.4 Kinetic Measurement

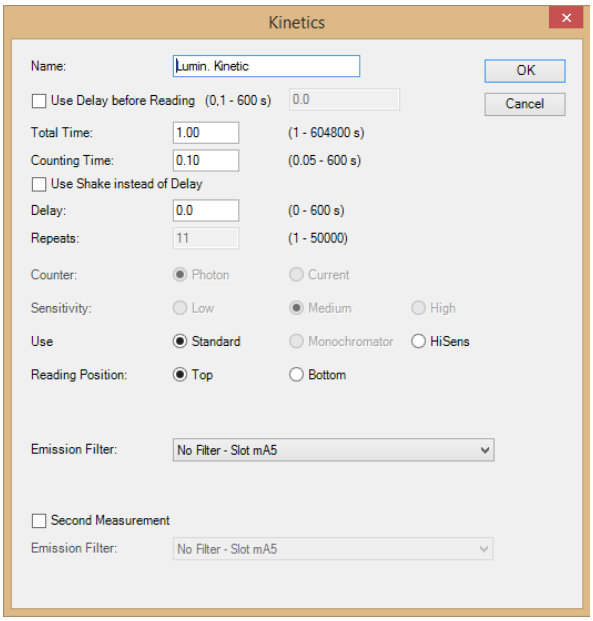
A kinetic measurement mode is appropriate for fast kinetics assays lasting over several seconds up to minutes, e.g. enzyme kinetics and Calcium influx

8.4.1 Defining a protocol for a kinetic measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Lumin. Kinetic*** for a luminescence kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay	0 to 600 sec
Repeats	(are calculated)
Reading Position	Choose reading from above (top) or below (Bot tom) the plate

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Emission Filter	usually: No Filter

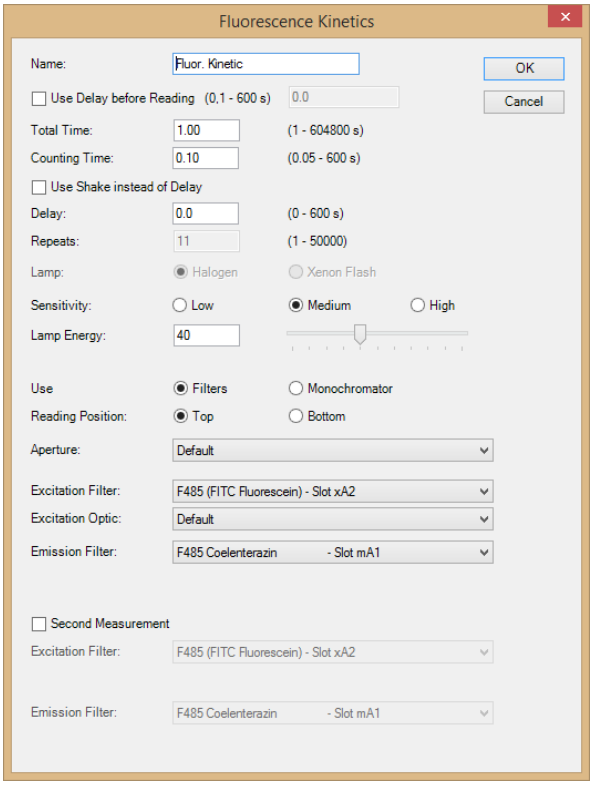
Note: filters must be defined prior in the Instrument menu

Second Measurement may be checked in case of ratiometric kinetics, e.g. in BRET applications

17. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

18. Double-click ***Fluor. Kinetic*** for a fluorescence kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Reading Position	Choose reading from above (top) or below (Bot tom) the plate

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Note: filters must be defined prior in the Instrument menu

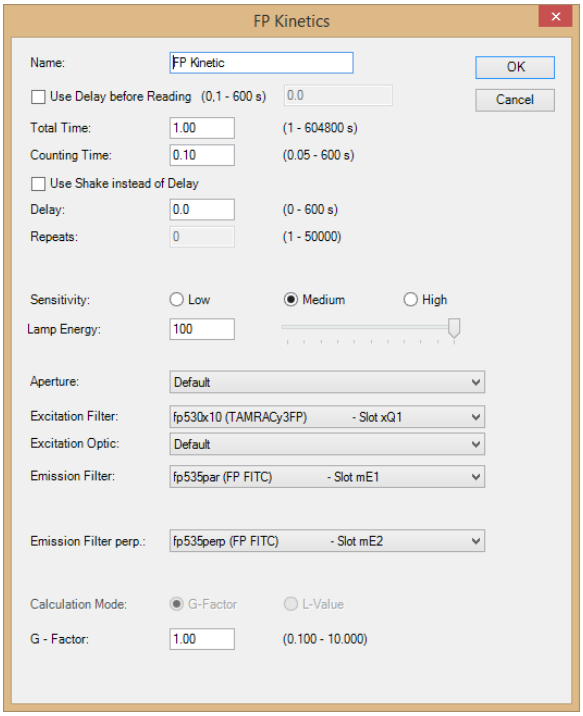
Operation Mode	by plate or by well

Second Measurement may be checked in case of ratiometric kinetics, e.g. in Calcium applications

19. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

20. Double-click ***FP Kinetic*** in the Fluorescence section for a fluorescence polarisation kinetic reading



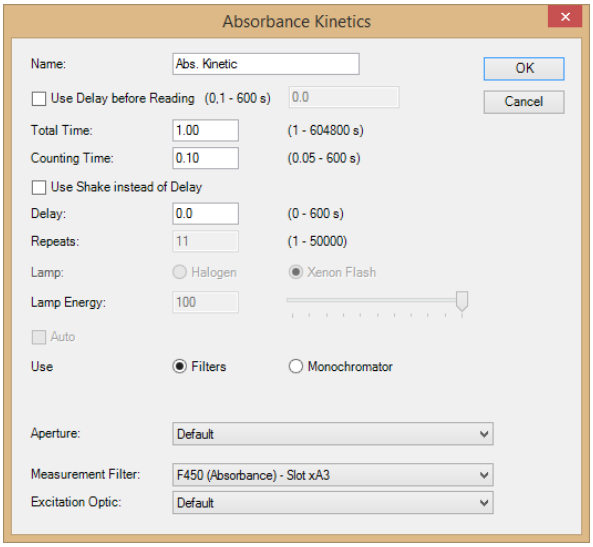
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Check Use Shake instead of Delay if needed	
Delay	0 to 600 s
Repeats	(are calculated)
Excitation Filter	choose the appropriate ex citation filter
Emission Filter	choose the appropriate emission filter for vertically oriented fluorescence
Emission Filter perp.	choose the appropriate emission filter for perpen dicularly oriented fluores cence

Note: FP filters use precisely aligned polarisation filters. For ideal results only use special FP fiter slides

Note: filters must be defined prior in the Instrument menu

G-Factor	Enter the correct G factor for your assay and this in strument derived from a G factor determination meas urement.
L-Value	Enter the correct L value for your assay and this in strument derived from a L value determination meas urement.
Operation Mode	by plate or by well

21. Double-click ***Abs. Kinetic*** for an absorbance kinetic reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

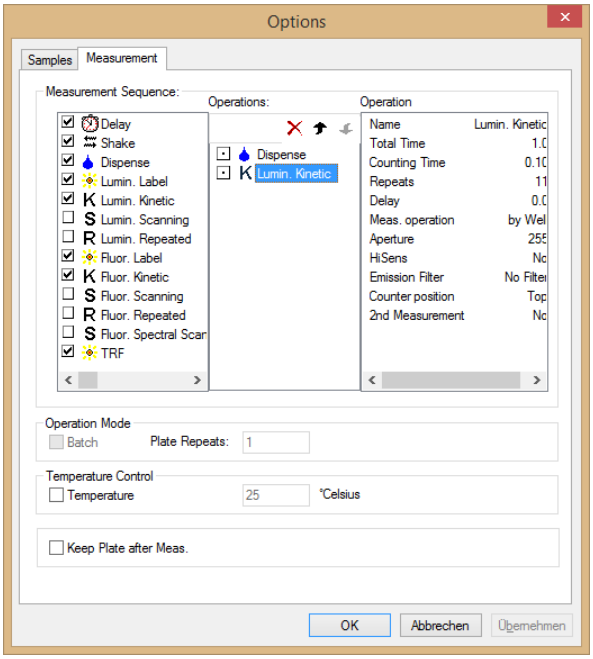
Use	Filters or Monochromator
Exc. Wavelength	set value
Exc. Slit Width	set value
Measurement Filter	select from the list

Note: filters must be defined prior in the Instrument menu

22. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

23. The **sequence of selected operations** will be displayed in the center column



Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

24. Check **Temperature** to activate the temperature control for this protocol
25. Define the **target temperature**
the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

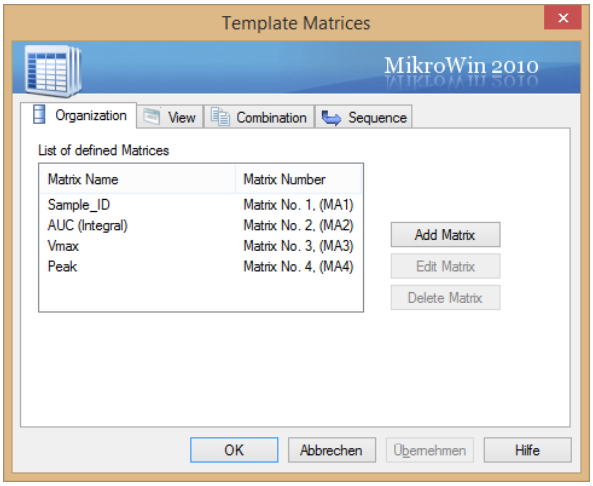
Robot, Barcode and Multi Plate Data File Mode are currently not active

26. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

27. Click **<OK>**

28. Click **<OK>** once more

For further calculations of the measurements follow the next steps:

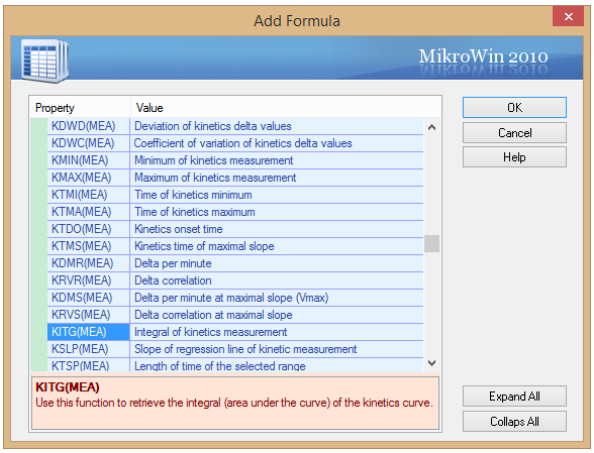


29. Select the **Template** tab and click **<Organization>** in the Matrices section. Click **<Add Matrix>**
30. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **AUC (Integral)**

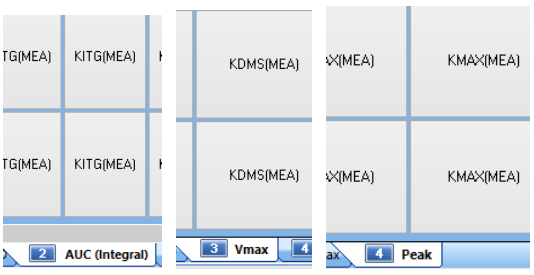
    Do the same for Matrix No. 3 (rename to e.g. **Vmax**) and Matrix No. 4 (rename to e.g. **Peak**)
31. Click **<OK>**

For export und print you have to refer to these matrices.

32. Change the view to **Calculation** in the **Matrices** section
33. Click on the 2 **AUC (Integral)** tab
34. Click **<Add Formula>** and expand **Kinetic calculation functions**
35. Select **KITG(MEA)** and click **<OK>**
36. Click the double-cross to assign for all wells



37. Proceed with the two other matrices alike:



3 **Vmax** **KDMS(MEA)**

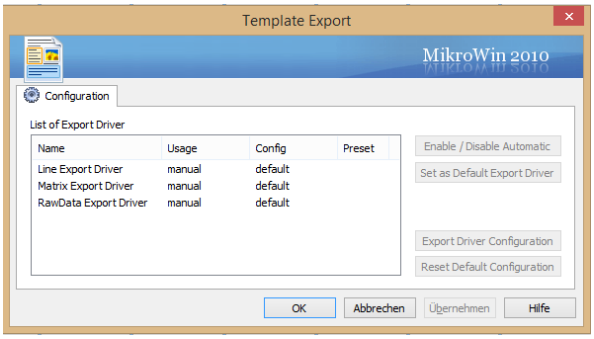
4 **Peak** **KMAX(MEA)**

When 2nd measurement has been checked MEA need to be replaced by LB1 and LB2 respectively and additional matrices may need to be created

Should you wish to define any additional calculations please refer to the Mikrowin manual.

22. To activate automatic export click **Configuration** in the **Export** section of the **Template** tab
23. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

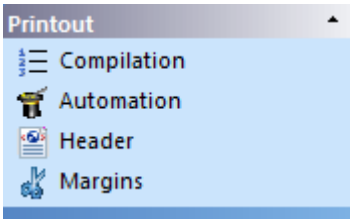


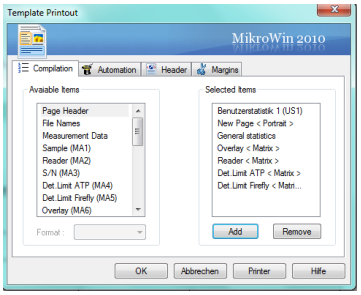
24. Click **<OK>**

55. Open the **Printout** section in the **Template** tab
56. Click **Compilation** to enter the following data

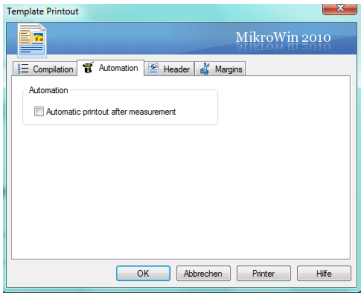
Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
first	measured data (matrix 2)*
second	measured data (matrix 3)*
ratio	ratio of readings (matrix 3)*
Gen. Statistics	measurement settings

* the selection and content depends on the matrix definition done in the Calculation section

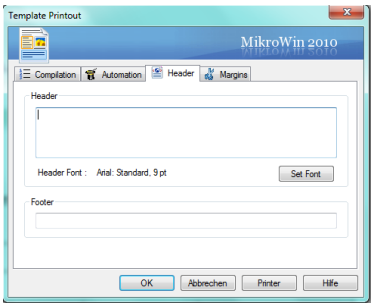




57. Check if **Automatic Print-out** is required



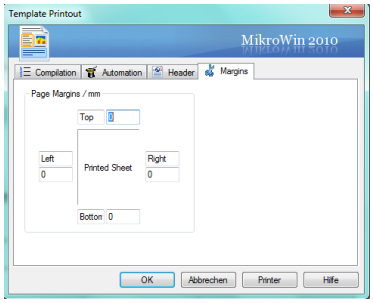
58. Type a **header** and/or **footer**

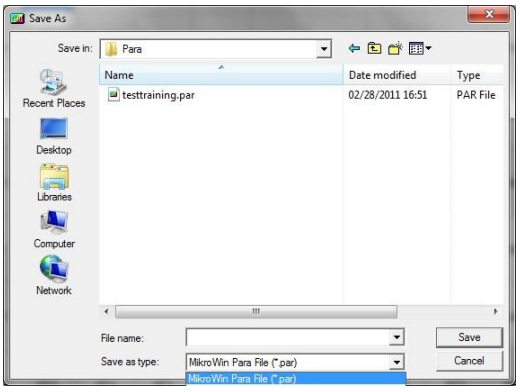


59. Select the page **margins** for the printout

60. Click **<OK>**

61. Go to **File | Para | Save As…**
62. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2S***
63. Select the file type **Mikrowin Para File (*.par)**
64. Type a meaningful **file name**
65. Click **<Save>**





Kinetic measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2 "Measurement with a single endpoint protocol"**.

8.5 Repeated Measurement

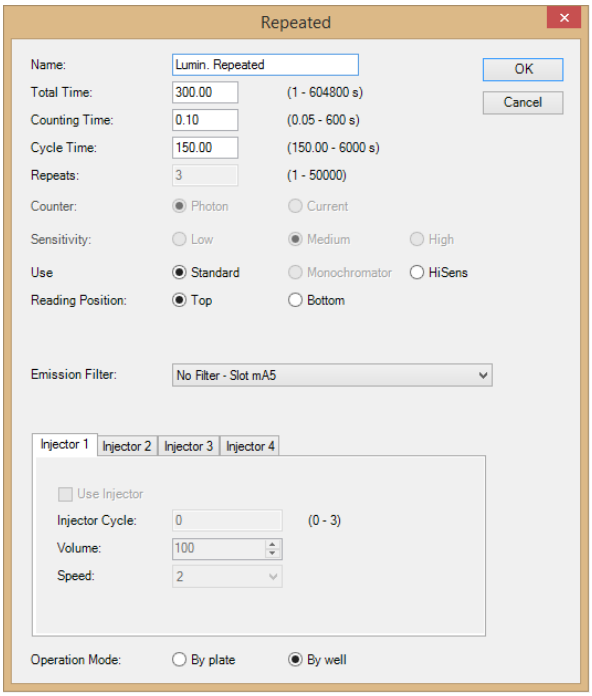
A repeated measurement mode is appropriate for long-term kinetic assays lasting over multiple minutes up to several days, e.g. cellular luminescence, slow enzyme kinetics, long-term gene expression or growth monitoring

8.5.1 Defining a protocol for a repeated measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Lumin. Repeated*** for a luminescence repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Reading Position	Choose reading from above (top) or below (Bottom) the plate.

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used.

Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

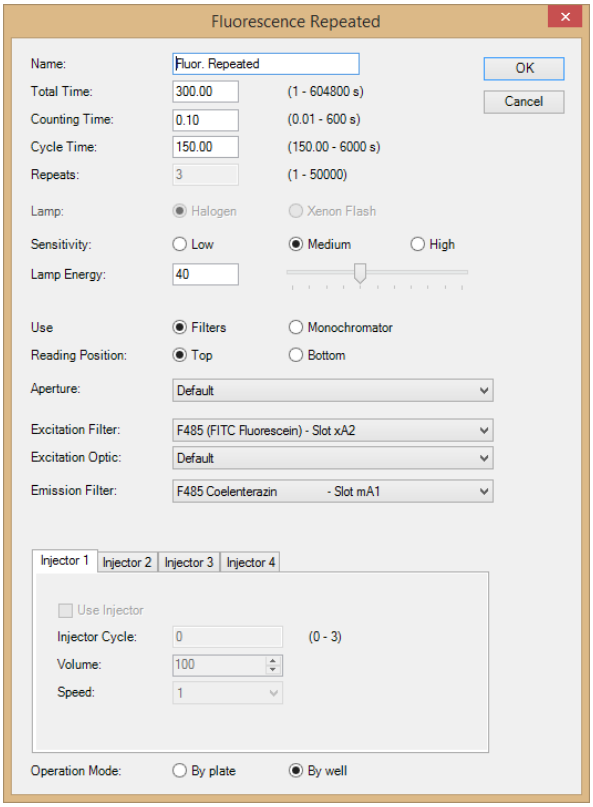
Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle	
Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

17. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications (BRET)

18. Double-click ***Fluor. Repeated*** in the Fluorescence section for a fluorescence repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Reading Position	Choose reading from above (top) or below (Bottom) the plate.

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used

Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Injector 1, …2, …3

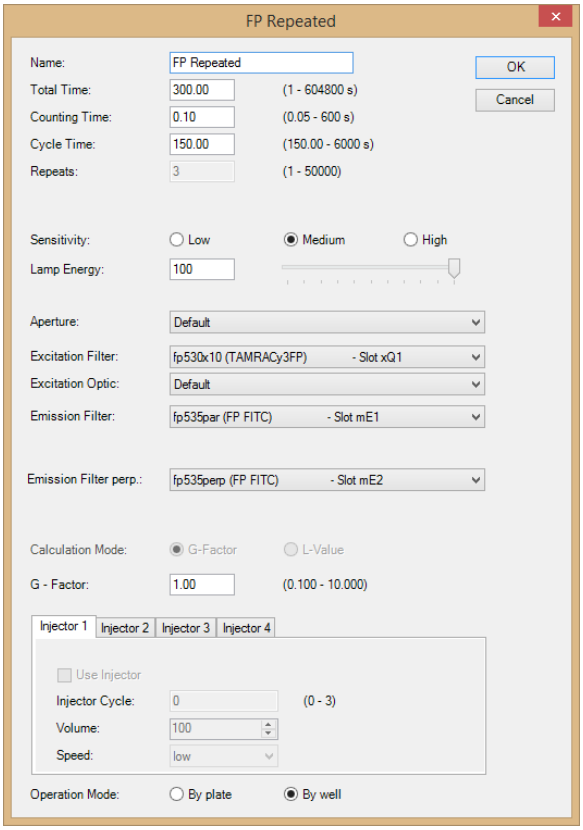
Check Use Injector for an injection within the repeated cycle

Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

19. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications (FRET)

20. Double-click ***FP Repeated*** in the Fluorescence section for a fluorescence repeated reading



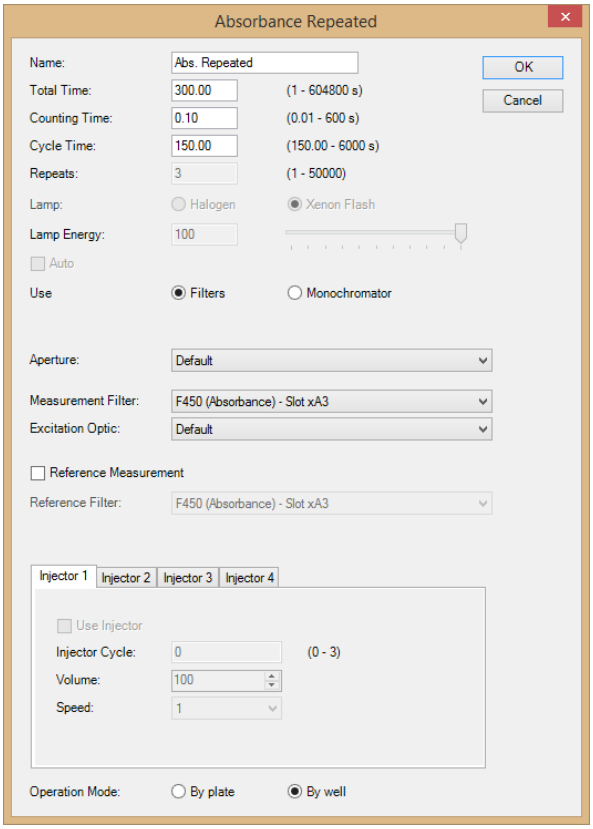
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Excitation Filter	choose the appropriate ex citation filter
Emission Filter	choose the appropriate emission filter for vertically oriented fluorescence
Emission Filter perp.	choose the appropriate emission filter for perpendicularly oriented fluorescence

Note: FP filters use precisely aligned polarisation filters. For ideal results only use special FP fiter slides

Note: filters must be defined prior in the Instrument menu

G-Factor	Enter the correct G factor for your assay and this instrument derived from a G factor determination measurement.
L-Value	Enter the correct L value for your assay and this instrument derived from a L value determination measurement.
Operation Mode	by plate or by well

21. Double-click ***Abs. Repeated*** in the Absorbance section for a absorbance repeated reading



Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecutive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Use	Filters or Monochromator
Exc. Wavelength	set value
Exc. Slit Width	set value
Measurement Filter	select from the list

Check Reference Measurement if needed

Reference Filter	select from the list

Note: filters must be defined prior in the Instrument menu

Injector 1, …2, …3

Check Use Injector for an injection within the repeated cycle

Injector Cycle	***0*** means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

22. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications

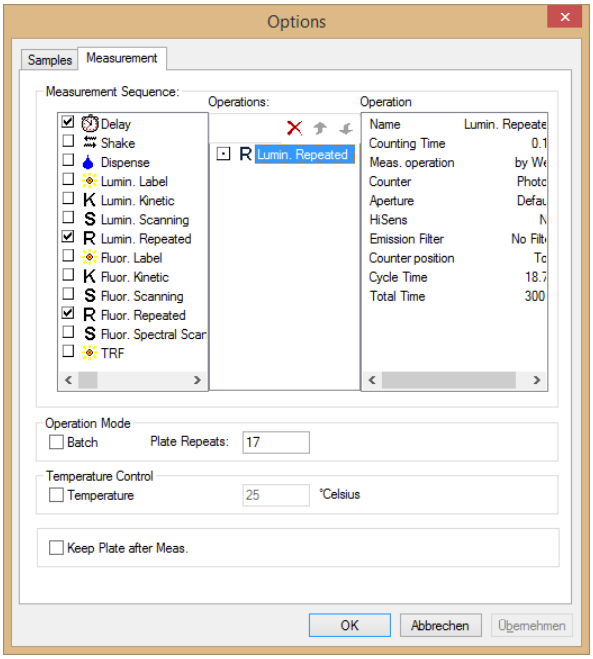
22. The **sequence of selected operations** will be displayed in the center column

    Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

    Operations can be deleted by highlighting and clicking the cross

    Details of the operation highlighted can be viewed on the right column

    An operation can be edited by double-clicking on it in the center column



23. Check **Temperature** to activate the temperature control for this protocol
24. Define the **target temperature**
    the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

    Robot, Barcode and Multi Plate Data File Mode are currently not active

25. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished

26. Click **<OK>**

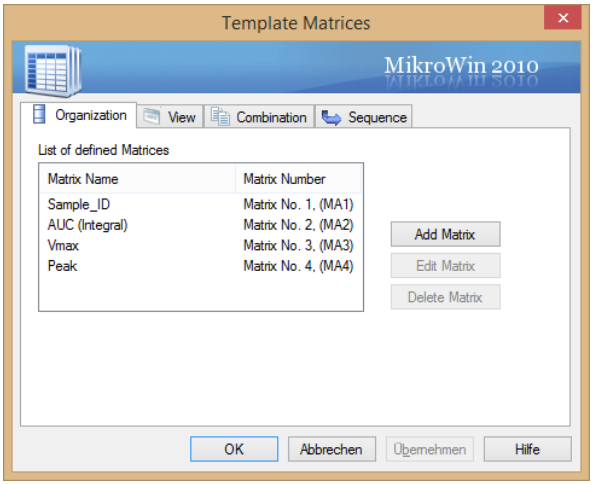
27. Click **<OK>** once more

    For further calculations of the measurements follow the next steps:

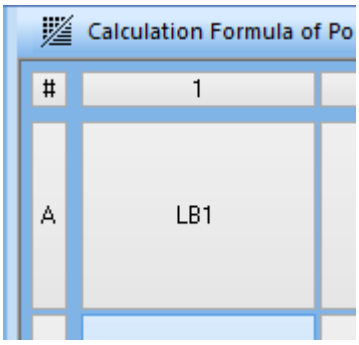
28. Select the **Template** tab and click **<Organization>** in the Matrices section. Click **<Add Matrix>**
29. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **AUC (Integral)**

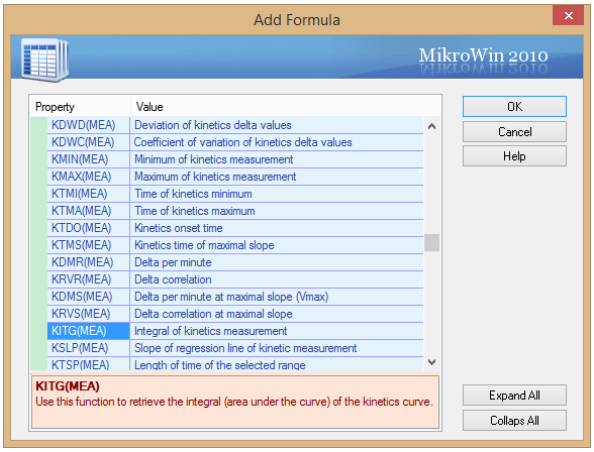
    Do the same for Matrix No. 3 (rename to e.g. **Vmax**) and Matrix No. 4 (rename to e.g. **Peak**)
30. Click **<OK>**

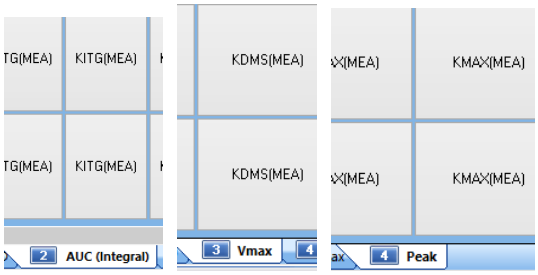
    For export und print you have to refer to these matrices.



38. Change the view to the **Calculation** section
39. Click on the 2 **AUC (Integral)** tab
40. Click **<Add Formula>** and expand **Kinetic calculation functions**
41. Select **KITG(MEA)** and click **<OK>**
42. Click the double-cross to assign for all wells







43. Proceed with the two other matrices alike:

3 **Vmax**	**KDMS(MEA)**
4 **Peak**	**KMAX(MEA)**

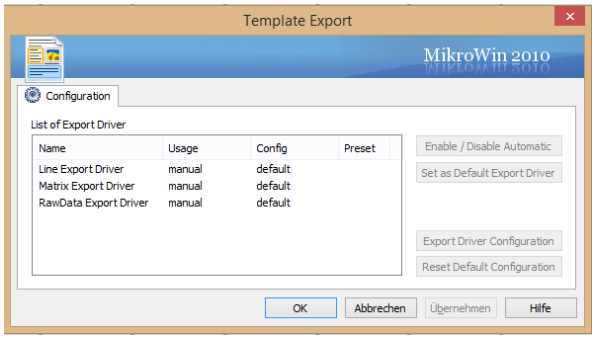
When 2nd measurement has been checked MEA need to be replaced by LB1 and LB2 respectively and additional matrices may need to be created

Should you wish to define any additional calculations please refer to the Mikrowin manual.

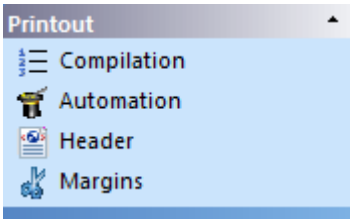
25. To activate automatic export click Configuration in the Export section of the Template tab
26. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

27. Click <OK>



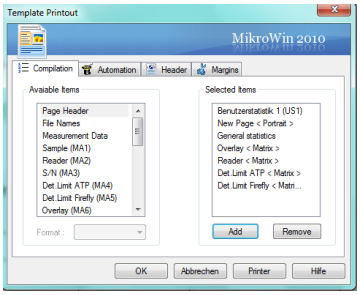
66. Open the **Printout** section in the **Template** tab
67. Click **Compilation** to enter the following data



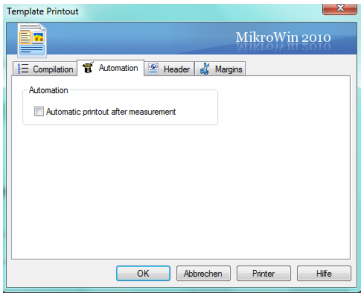
Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
first	measured data (matrix 2)*

second	measured data (matrix 3)*
ratio	ratio of readings (matrix 3)*
Gen. Statistics	measurement settings

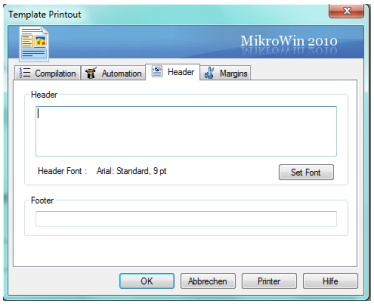
* the selection and content depends on the matrix definition done in the Calculation section



68. Check if **Automatic Print-out** is required

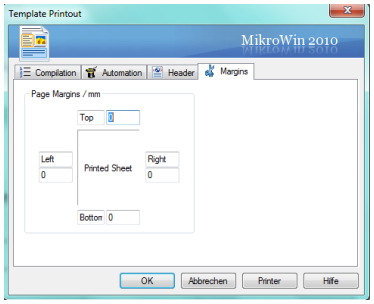


69. Type a **header** and/or **footer**

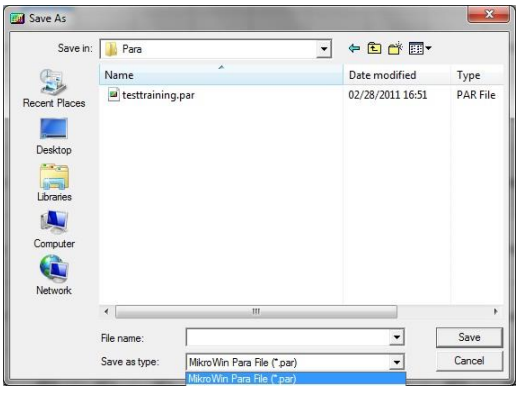


70. Select the page **margins** for the printout

71. Click **<OK>**



72. Go to **File | Para | Save As…**
73. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2S***
74. Select the file type **Mikrowin Para File (*.par)**
75. Type a meaningful **file name**
76. Click **<Save>**



8.5.2 Repeated measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2 "Measurement with a single endpoint rotocol"**.

8.6 Scanning Measurement

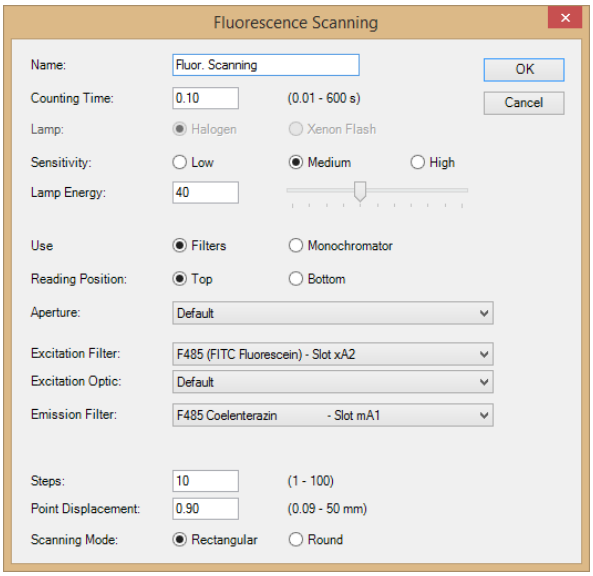
A scanning measurement mode is appropriate for assays with heterogeneous distribution of signal, e.g. cellular assays

8.6.1 Defining a protocol for a scanning measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click ***Fluor. Scanning*** for a fluorescence scanning reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Reading Position	Choose reading from above (top) or below (Bot tom) the plate.

Note: If reading position ***Bottom*** is selected, the red plate frame and the "*mH*" Emission filter slide must be used

Use	Filters or Monochromator
Excitation Filter	select from the list
Exc. Wavelength	set value
Exc. Slit Width	set value
Emission Filter	select from the list

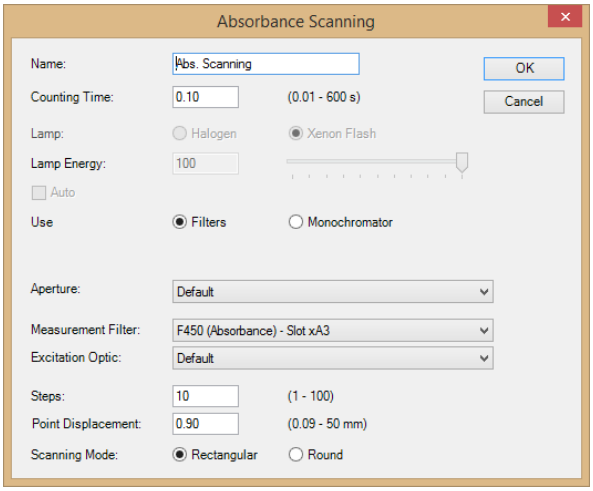
Note: filters must be defined prior in the Instrument menu

Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

17. Click **<OK>**

18. Double-click ***Abs. Scanning*** for a absorbance scanning reading



Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or ***Auto***

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Use	Filters or Monochromator
Exc. Wavelength	set value
Exc. Slit Width	set value
Measurement Filter	select from the list

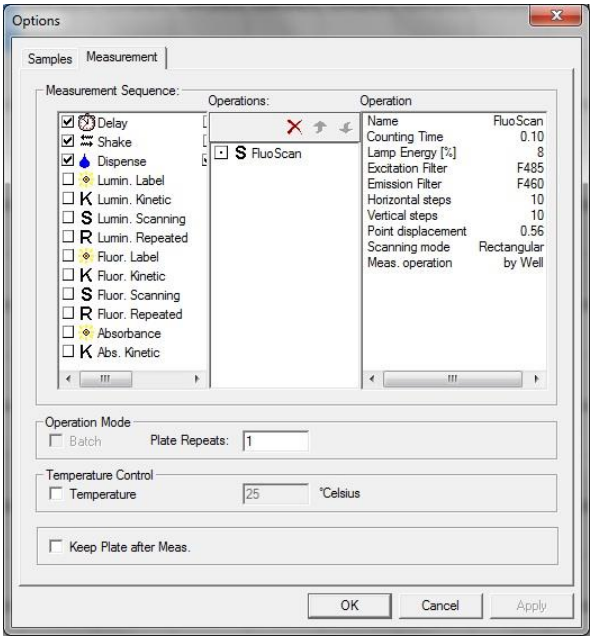
Note: filters must be defined prior in the Instrument menu

Steps	1 to 100 scanning points in one direction, the other direction will have the same amount of points
Point Displacement	distance between points

Select rectangular or round matrix

19. Click **<OK>**

20. The **sequence of selected operations** will be displayed in the center column



Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

21. Check **Temperature** to activate the temperature control for this protocol
22. Define the **target temperature**
the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

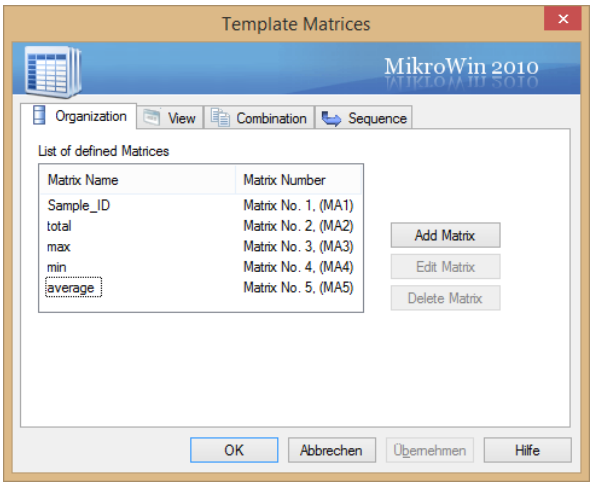
23. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the in-

strument after the reading being finished

24. Click **<OK>**

25. Click **<OK>** once more

For further calculations of the measurements follow the next steps:

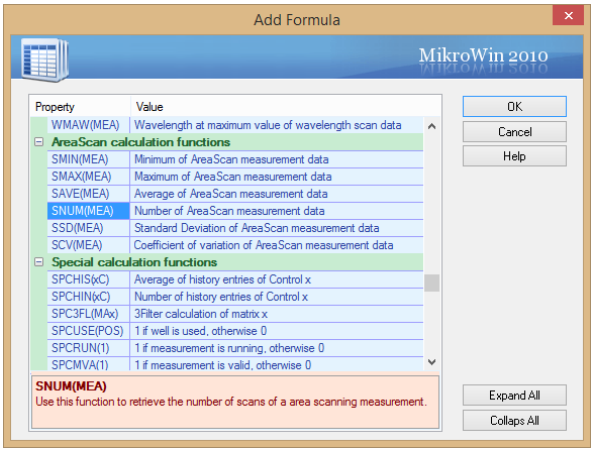


26. Select the **Template** tab and click **<Organization>** in the Matrices section. Click **<Add Matrix>**
27. click **<Add Matrix>** again
28. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **total**

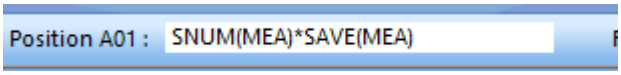
    Do the same for Matrix No. 3 (rename to e.g. **max**), Matrix No. 4 (rename to e.g. **min**) and Matrix No. 5 (rename to e.g. **average**)
29. Click **<OK>**

For export und print you have to refer to these matrices.

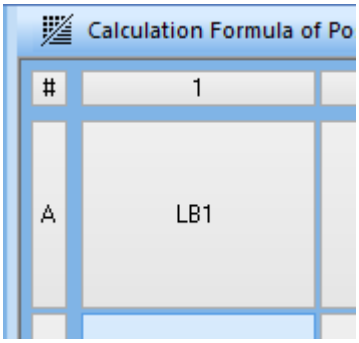
30. Change the view to the **Calculation** section

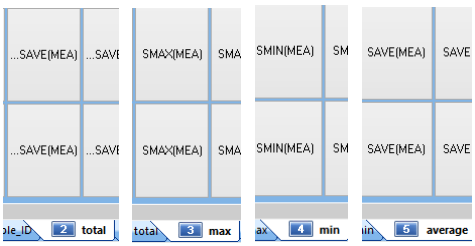


31. Click on the 2 **total** tab
32. Click **<Add Formula>** and expand **Area Scan functions**
33. Select **SNUM(MEA)** and click **<OK>**
34. Type an **asterisk (*)**
35. Click **<Add Formula>** and expand **Area Scan functions**
36. Select **SAVE(MEA)** and click **<OK>**



37. Click the double-cross to assign for all wells





38. Proceed with the three other matrices alike:

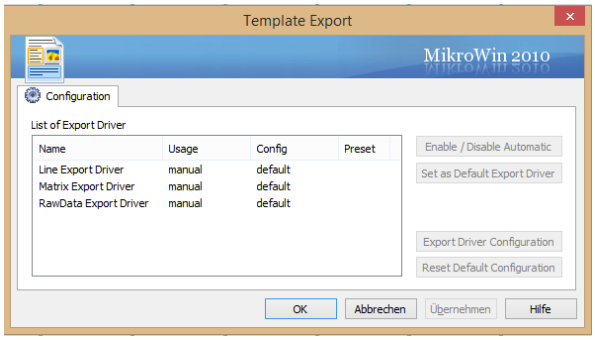
    3 **max** SMAX(MEA)

4 min	SMIN(MEA)
5 average	SAVE(MEA)

Should you wish to define any additional calculations please refer to the Mikrowin manual.

28. To activate automatic export click **Configuration** in the **Export** section of the **Template** tab
29. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

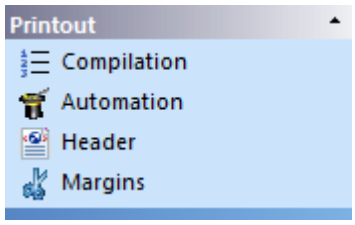


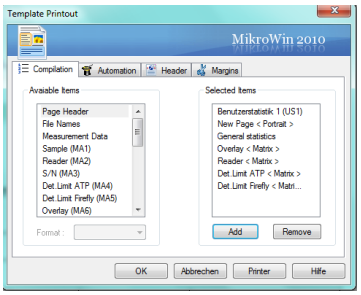
30. Click **<OK>**

77. Open the **Printout** section in the **Template** tab
78. Click **Compilation** to enter the following data

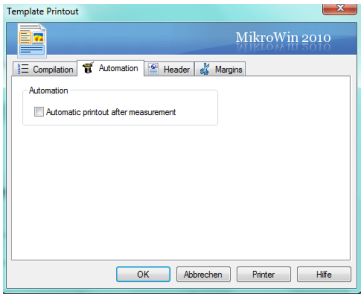
Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
first	measured data (matrix 2)*
second	measured data (matrix 3)*
ratio	ratio of readings (matrix 3)*
Gen. Statistics	measurement settings

* the selection and content depends on the matrix definition done in the Calculation section

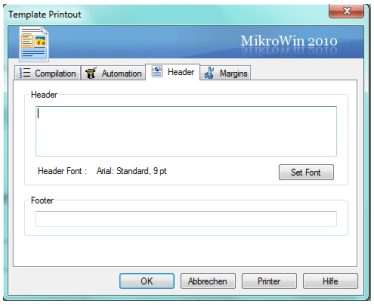




79. Check if **Automatic Print-out** is required

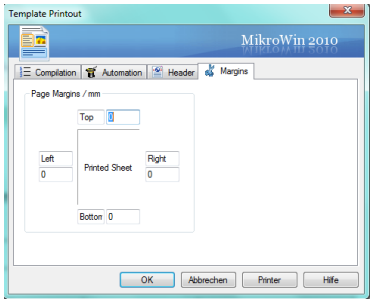


80. Type a **header** and/or **footer**

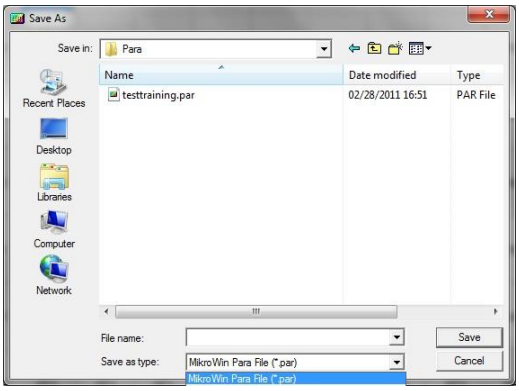


81. Select the page **margins** for the printout

82. Click **<OK>**



83. Go to **File | Para | Save As…**
84. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2S***
85. Select the file type **Mikrowin Para File (*.par)**
86. Type a meaningful **file name**
87. Click **<Save>**



8.6.2 Scanning measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2 "Measurement with a single endpoint rotocol"**.

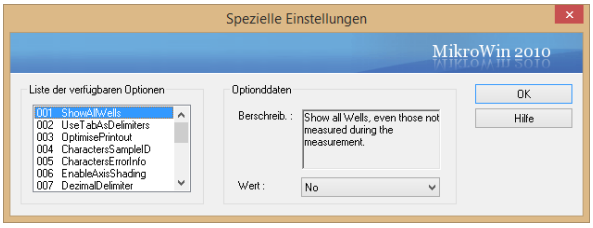
8.7 Measurement with curve fitting

A raw data measurement that contains standards with known concentrations which are used to determine unknown concentrations of the samples.

8.7.1 Defining a protocol with curve fitting

When working with Blank subtraction it is recommended to change a global setting in Mikrowin first.

1. Go to **Installation | Settings** and hit the **ALT** and the **S** keys
2. Scroll to **014 DisableAutoBlankSubtraction**
3. Select the value **Yes**
4. Click **<OK>**
5. Click **<OK>** once more



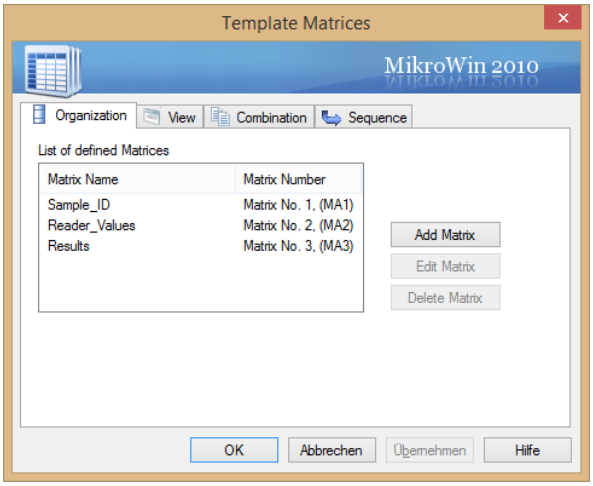
Next, follow the instructions until step 29 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

For further calculations of the measurements follow the next steps:

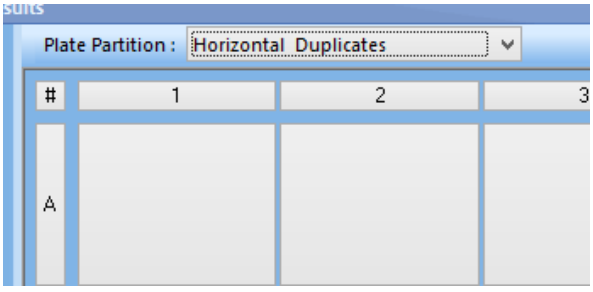
30. Select the **Template** tab and click **<Organization>** in the Matrices section. Click **<Add Matrix>**
31. Highlight Matrix No. 3, click **<Edit Matrix>** and rename to e.g. **Blank subtr**

    Do the same for Matrix No. 4 (rename to e.g. **Conc**) and Matrix No. 5 (rename to e.g. **Average**)
32. Click **<OK>**

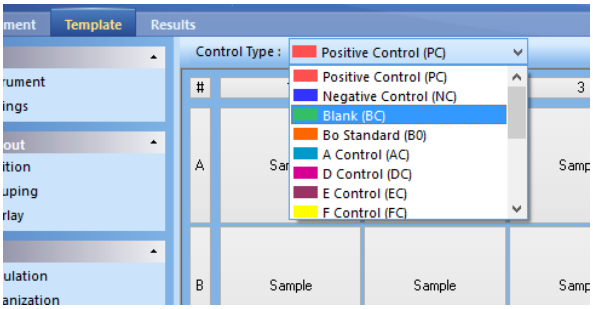


For export und print you have to refer to this matrices.

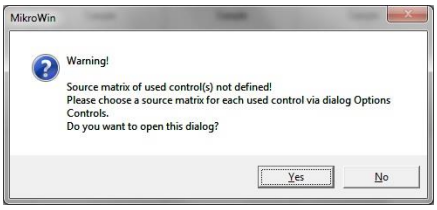
33. Change the view to the **Template** tab.
34. Click on **Partition** in the **Plate layout** section and select a pattern matching your replicates in the **Plate Partition** drop box
35. Click the double-cross to assign for all wells



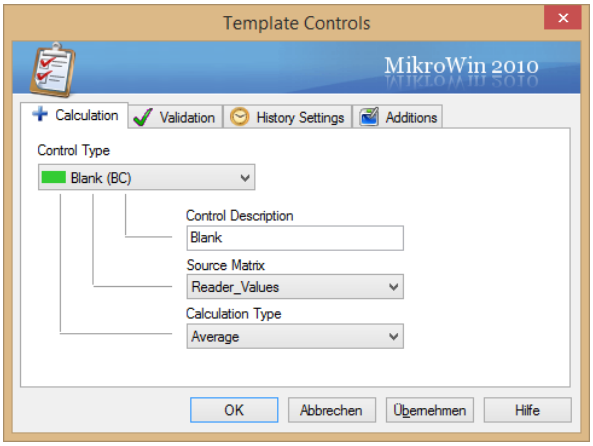
36. Go to the **Controls** section and click **<Control Positions>**
37. Select **Blank (BC)** in the **Control Type** drop box
38. Click into wells **A1/A2** to assign the blank to these wells



39. Click **<Yes>** in ***Warning! Source matrix***… dialogue



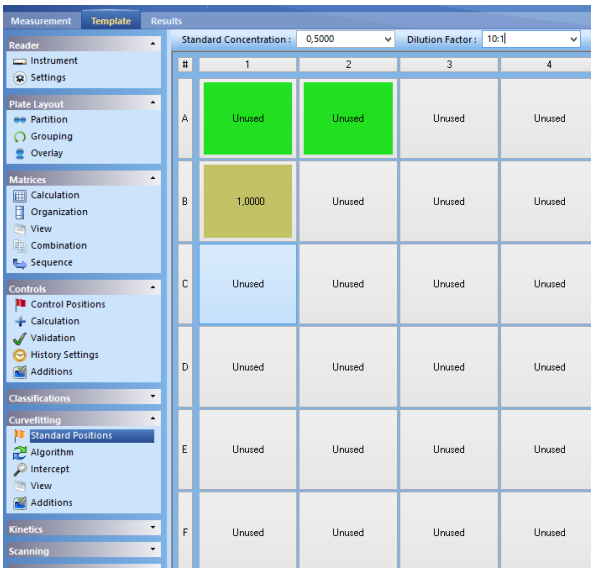
40. Select **Reader_Values** in **Source Matrix** drop box

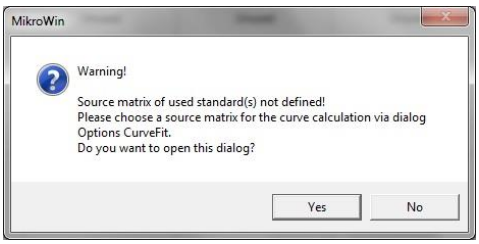


41. Select the **Template** tab and click **<Standard positions>** in the **Curvefitting** section.
42. Click into **Standard Concentration** field and enter the concentration of the first standard
43. Select the matching dilution in the **Dilution Factor** drop box
44. Click into B1/B2 (resp. the set of wells containing the **first standard concentration**) and drag the mouse to the set of wells with the last standard concentration

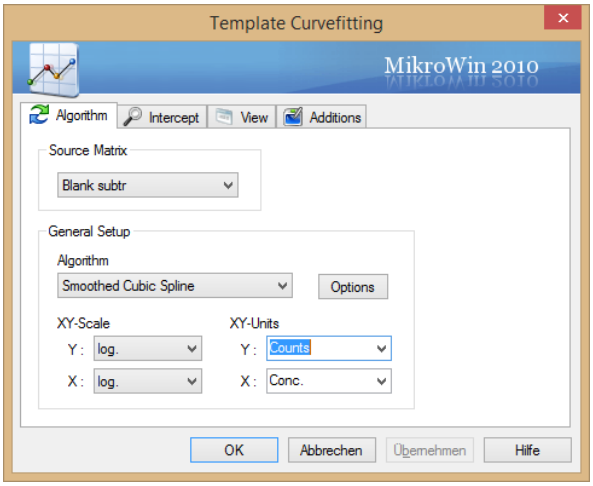
    In case you work with non-regular concentration series, click into the first set of wells, enter the concentration and hit the **ENTER** key

    Go ahead until the last concentration is being entered

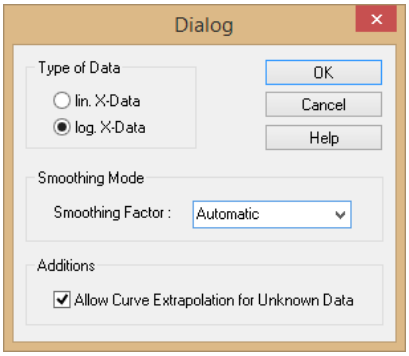




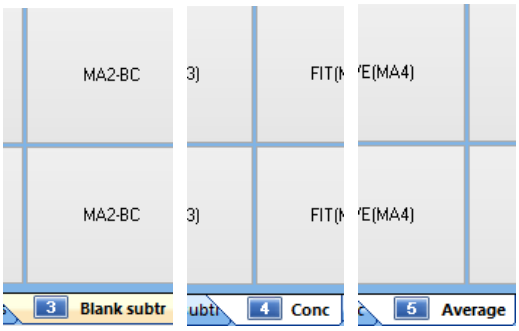
45. Click **<Yes>** in ***Warning! Source matrix***… dialogue



46. Select the **Source Matrix**, e.g. **Blank Subtr**
47. Select the curve fit **Algorithm**, e.g. **Smoothed Cubic Spline**
48. Define the **X and Y axis scales**, e.g. log for both
49. Type or select the axes **Units**
50. Click on **<Options>**



51. Select the **Type of Data**, whether they span a linear or a logarithmic range
52. **Smoothing Factor** can be kept as Automatic
53. **Curve Extrapolation** may be checked
54. Click **<OK>**
55. Click **<OK>** once more
56. Change the view to **Calculation** in the **Matrices** section
57. Click on the ***3*** **Blank subtr** tab

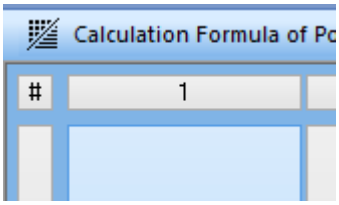


58. Type **MA2 - BC** into the Calculation Formula:

    MA2 = matrix 2 = contains the reader values

    BC = Blank Control

59. Click the double-cross to assign for all wells



60. Proceed with the two other matrices alike:

    4 **Conc** **FIT(MA3)**

    FIT = curve fitting

    MA3 = matrix 3 = the fit concentration calculation is applied to the values of matrix 3

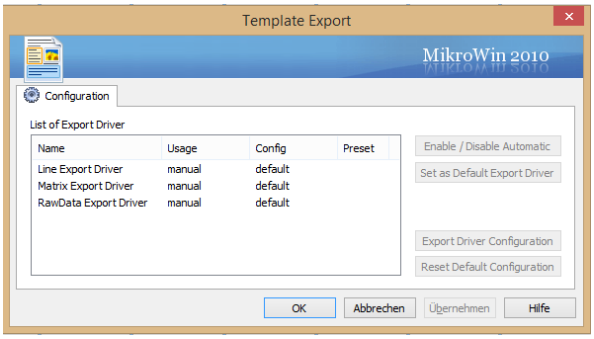
    5 **Average** **AVE(MA4)**

AVE = calculation of mean value

MA4 = matrix 2 = calculation is done on the values of matrix 4 (in this case the concentrations)

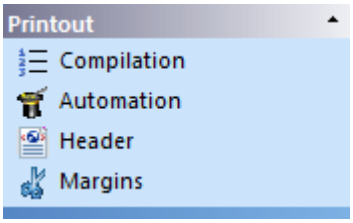
Should you wish to define any additional calculations please refer to the Mikrowin manual.

31. To activate automatic export click Configuration in the Export section of the Template tab
32. Select the appropriate and pre-defined export driver



The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

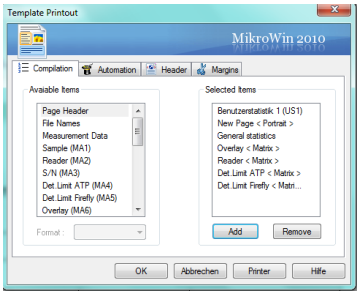
33. Click <OK>



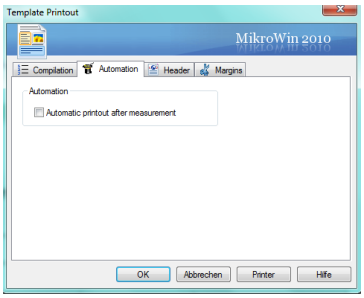
88. Open the **Printout** section in the **Template** tab
89. Click **Compilation** to enter the following data

Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
first	measured data (matrix 2)*
second	measured data (matrix 3)*
ratio	ratio of readings (matrix 3)*
Gen. Statistics	measurement settings

* the selection and content depends on the matrix definition done in the Calculation section

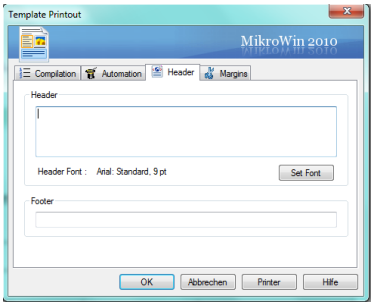


90. Check if **Automatic Print-out** is required

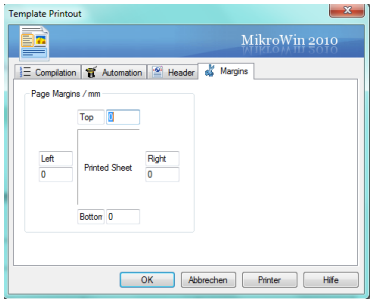


91. Type a **header** and/or **footer**

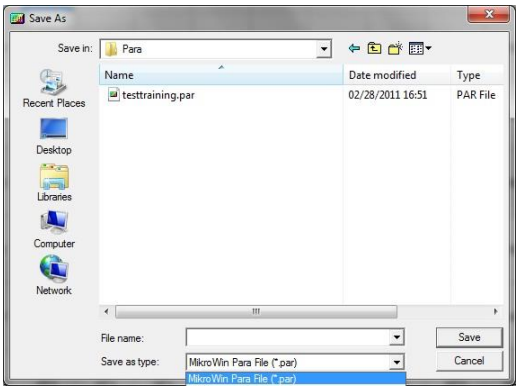
92. Select the page **margins** for the printout



93. Click **<OK>**



94. Go to **File | Para | Save As…**
95. Create and/or Select an appropriate directory, e.g. ***ParaTriStar2S***
96. Select the file type **Mikrowin Para File (*.par)**
97. Type a meaningful **file name**
98. Click **<Save>**



8.7.2 Measurement with a Curvefit parameter file

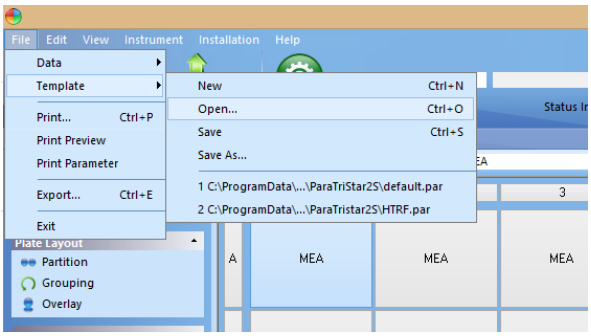
The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

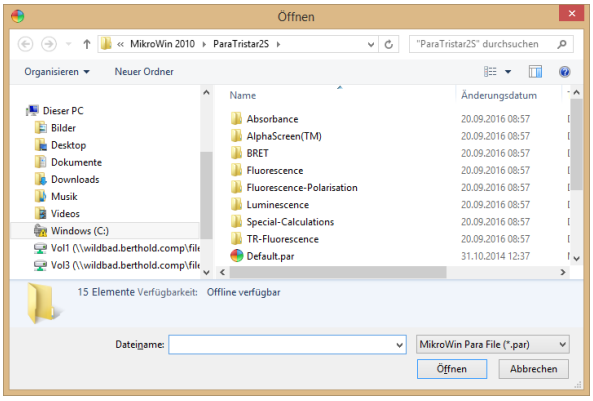
Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See chapter 9 of this manual.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

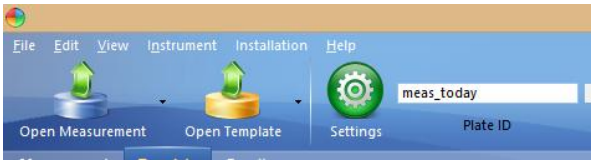
Note: Make sure the appropriate plate frame is inserted

21. Click **Open** in the **File/Template** menu
22. Select **File of type**: Mikrowin Para File
23. Select the appropriate file
24. Click **<Open>**





25. Enter a **file name** under which the measurement is to be stored



26. Click **<Start>**

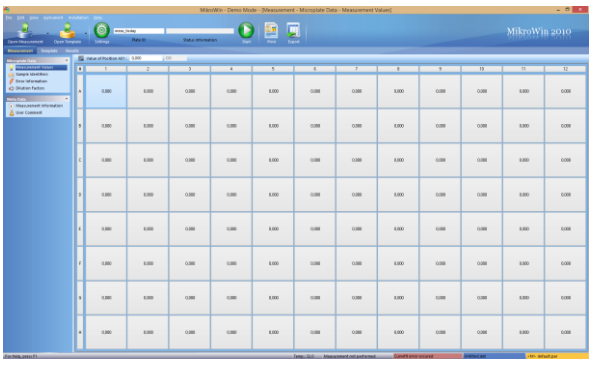
27. Insert the **microplate** with your samples:
well A1 facing the rear and left

    Use the **black frame** for microplates with plate heights of 15 mm (±1 mm), e.g. 96 and 384 well plates

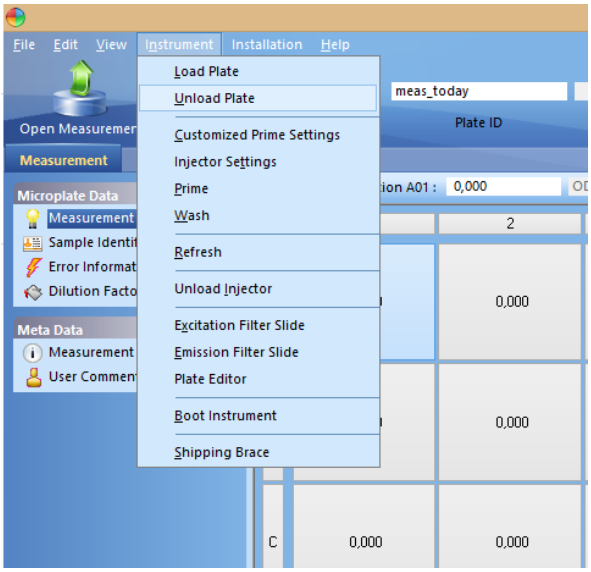
    Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates and for bottom reading.

28. Click **<OK>**

29. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed



30. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument

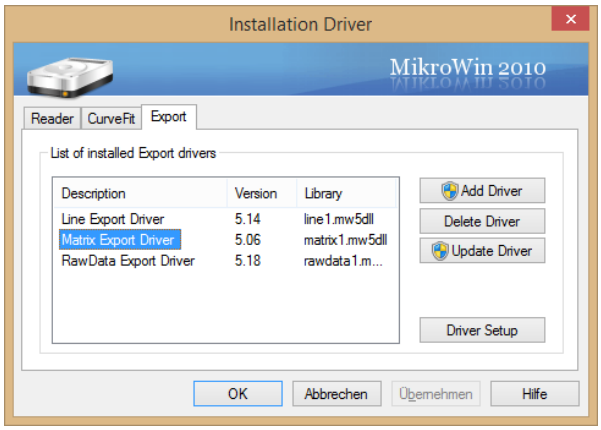


8.8 Export and print-out in Mikrowin

The export of (calculated) results and raw (measurement) data is carried out via export drivers. The export drivers have to be installed and configured. Data can be exported in XLS and TXT file formats.

8.8.1 Export Driver Configuration

Export drivers have to be installed if you want to export data. In addition, you have to set up the export driver and you have to specify data structure, data matrices as well as header and footer. Data is exported depending on the driver selected and configured in this dialog box. To use another data format, you can select another driver before running a measurement or set up the selected driver new.
In the **Installation Driver** dialog box, select the **Export** tab to view the available drivers. You may choose:



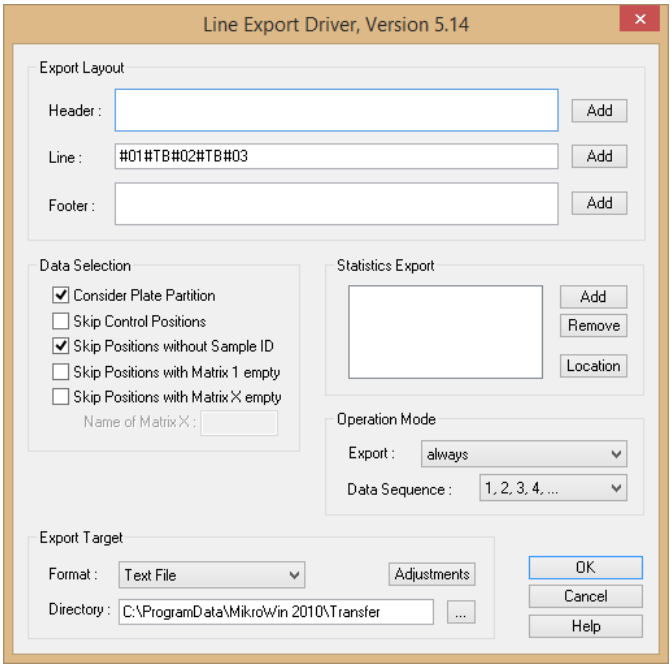
Selection of export drivers

Line Export Driver Driver (template) for export of calculated data with list (i.e. table-type) structure. Only data that are visible on result matrices can be exported. File formats may be EXCEL, Text (ASCII) and CSV.

Matrix Export Driver Driver (template) for export of calculated data with matrix (i.e. plate lay-out) structure. Only data that are visible on result matrices can be exported. File formats may be EXCEL, Text (ASCII) and CSV.

RawData Export Driver Driver (template) for export of all raw data. File formats may be EXCEL, Text (ASCII) and CSV. Whether data in the export file are presented in list or matrix format depends on the settings and data origin.

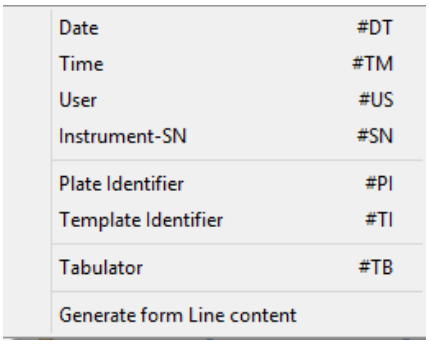
Line Export Driver Select this export driver to define a table-type file. Parameters (header, matrix and footer as well as the target directory for data storage) are entered in the same manner as for an matrix-type file (see previous section).



Line export driver setup

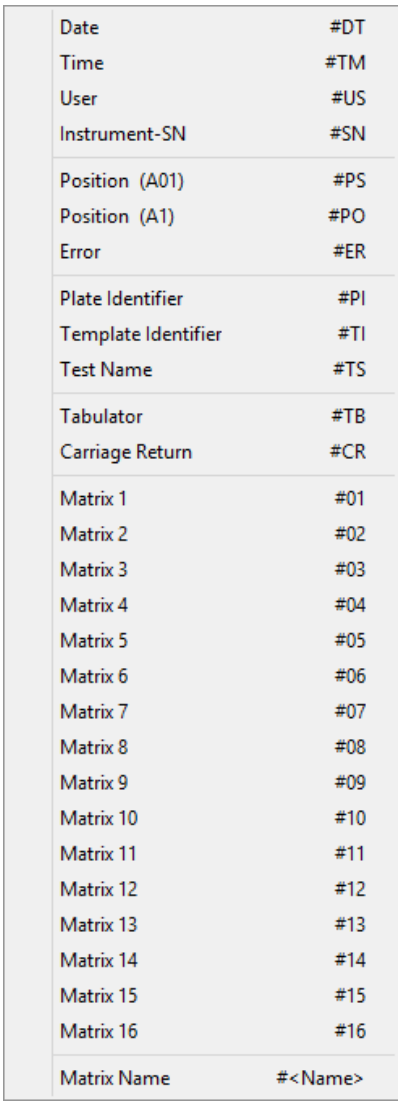
Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).



Context menu for entering header placeholders

Line In this text box you enter the matrices whose data you wish to export. In general, one exports only data from the result matrices. ***Make sure*** that the matrices and their numbers specified here is identical with the number of the result matrix in the parameter file.



*Context menu **Line Export Driver***

Click <**Add**> to open the context menu and select the matrix number (1 – 15) or define the matrix name. Several matrices can be selected one after the other. They are entered in the matrix list.
Click <**Delete**> to delete the selected matrix from the matrix list.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.

Data Selection Define additional options regarding data sources and positioning.

Consider Plate Partition This option should be checked when replicates are used and they are to be exported next to each other.

Skip Control Positions This option may be checked if values of Controls are not supposed to be exported.

Skip Positions without Sample ID Check if only samples with sample IDs are to be exported.

Skip Empty Positions of Matrix 1 This option may be used if the values of unused wells are not to be exported. Matrix 1 must contain an appropriate variable like **MEA** or **LB 1**.

Operation Mode Define additional options.

Export The proper setting is **Always**.

Add General Statistics Options are **Yes** or **No**.

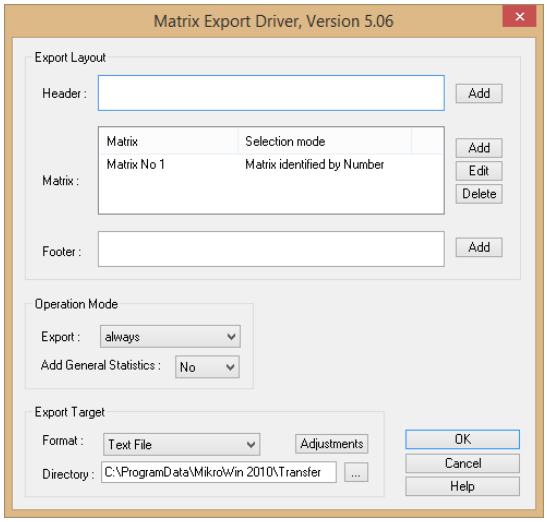
Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from **Text File, XLS File, CSV File, CommPort and Clipboard**.

Target Define the directory which the file is to be exported to. You may use the browse <**…**> button to locate an appropriate directory.

Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

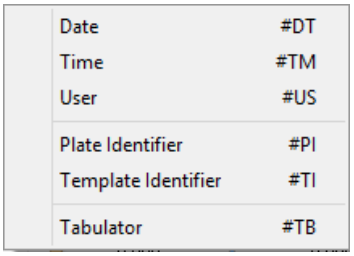
Matrix Export Driver If you select the Matrix export driver, you have to define the following configuration:



Matrix export driver setup

Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).



Context menu for entering header placeholders

Example:
Header with date, time and plate identification, separated by tab characters: **#DT#TB#TM#TB#PI**

Matrix In this text box you enter the matrices whose data you wish to export. In general, one exports only data from the result matrices. ***Make sure*** that the matrices and their numbers specified here is identical with the number of the result matrix in the parameter file.

Click <**Add**> to open the context menu and select the matrix number (1 – 15) or define the matrix name. Several matrices can be selected one after the other. They are entered in the matrix list.

Click <**Delete**> to delete the selected matrix from the matrix list.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.

Operation Mode Define additional options.

Export The proper setting is **Always**.

Add General Statistics Options are **Yes** or **No**.

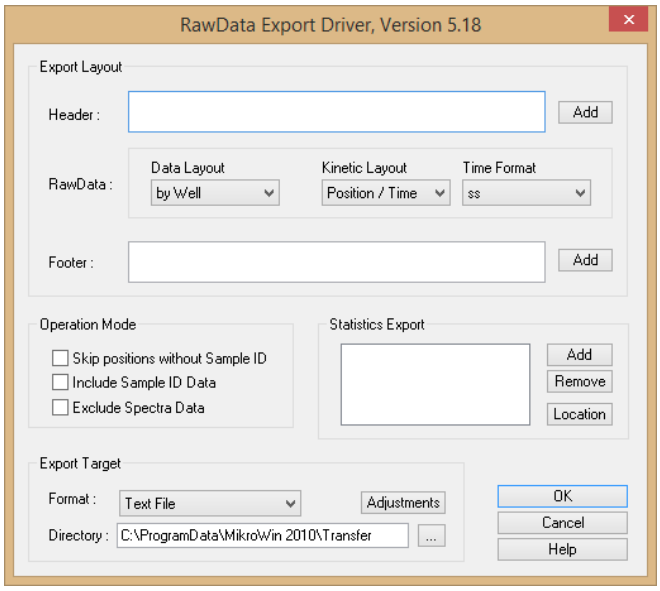
Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from **Text File, XLS File, CSV File, CommPort and Clipboard**.

Target Define the directory which the file is to be exported to. You may use the browse <**…**> button to locate an appropriate directory.

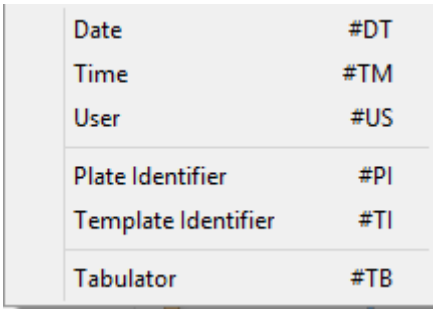
Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

RawData Export Driver With this export driver a file containing all raw data will be created.
For the export of kinetic data the kinetic layout can be selected (see below).
When the Rawdata Export driver is used for values derived from scanning operations each well is displayed in a separate area with the individual reading points displayed in an X-Y matrix representing the scanning positions.
With data coming from multilabel measurements (e.g. BRET) with a single reading per wavelength the data are exported in a respective amount of matrices representing the plate layout.
Data from multi-plate readings (Batch mode) are also exported in a matrix orientation. ***Note:*** only single readings are supported!



Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).



Context menu for entering header placeholders

RawData Kinetik Layout
The selection ***Position/Time*** has a column adressed to each well position (left to right) and the consecutive readings are entered in lines (down).
The selection ***Time/Position*** has a line adressed to each well position (down) and the consecutive readings are entered in columns (left to right).

Note: Keep in mind that EXCEL supports a maximum of 256 columns.

Time Output Format
Select the time format a kinetic reading. Choices are: ***hh:mm:ss*** or ***sec.msec***.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.

Operation Mode Define additional options.

Add General Statistics Options are **Yes** or **No**.

Add Sample ID information Check if you want that information added to each value.

Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from **Text File, XLS File, CSV File, CommPort and Clipboard**.

Target Define the directory which the file is to be exported to. You may use the browse <**…**> button to locate an appropriate directory.

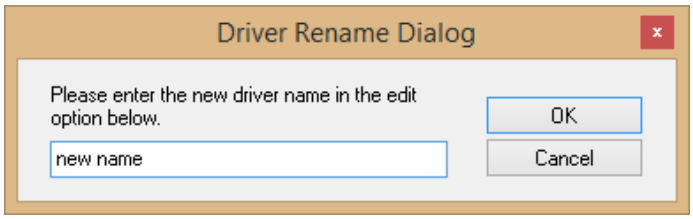
Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

8.8.2 Adding additional / replicating export drivers

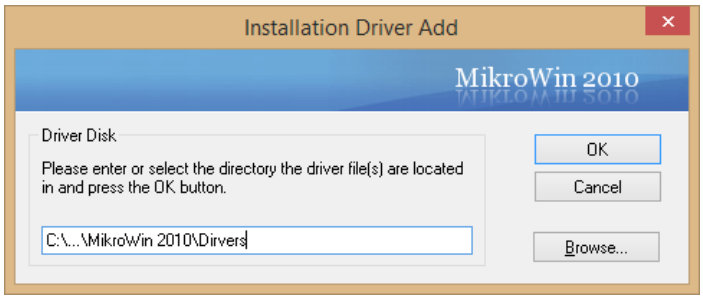
Especially in multi-user environment the individual users will have their own demands for export driver setups. To support this multiple copies of the export driver can be installed and each of the copies can be individually set up.

It is recommended for convenience and security to create a new directory within the ***Mikrowin 2010*** directory (e.g. called "**Drivers**") and copy the original export drivers ***matrix1.xdl***, ***line1.xdl*** and ***rawdata1.xdl*** to it.

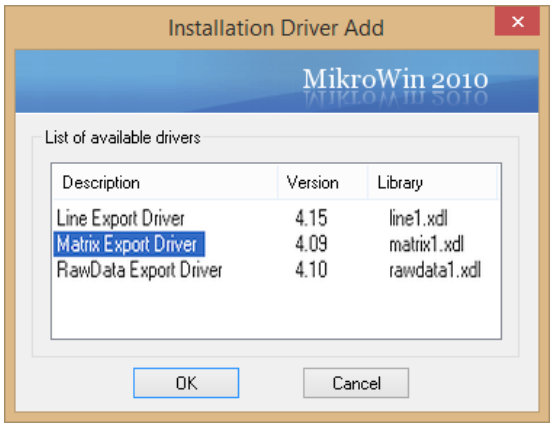
First, rename the export driver that is to be installed a second time by highlighting it in the **Installation | Driver | Export** menu. Hit the **ALT** and the **R** keys simultaneously. You can enter a new name for the driver. Confirm with <**OK**>.



Now you can re-install the driver again by the clicking <**Add Driver**> and browsing to the driver directory you created.



Select the respective driver in the dialogue displayed.



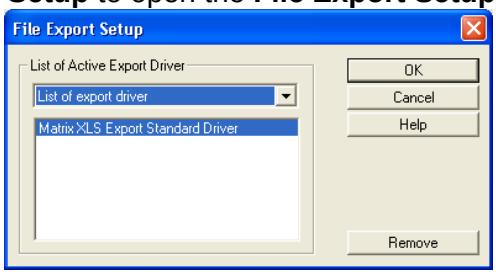
You may repeat this procedure as often as necessary to get an appropriate number of export drivers.

8.8.3 Automatic export

Choose the menu item **File | Export Setup** to select the export driver that is to be loaded automatically upon successful completion of a reader run. If a driver has been selected for the active parameter file, data evaluation is performed after completion of the respective measurement and data export is carried out in accordance with the selected driver.

Please keep in mind:
This function is only valid for the active parameter file.
Prerequisite for automatic data export is that the respective export driver has been installed and set up in the menu item **Installation | Driver** (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**) and the export driver has been selected in the menu item **File | Export** (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**).

Open parameter file.
Select **File | Export Setup** to open the **File Export Setup** dialog box.



File Export Setup dialog box

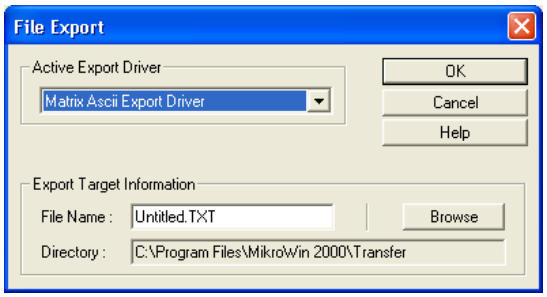
List of Active Export Driver
Select the export driver you want to use for automatic data export upon successful completion of a reader run. Click on the arrow button to open the list showing the available drivers and select the driver you want. The selected drivers appear in the text box directly below the drop-down list box.
To delete a driver from the list, select this driver and then click <**Remove**>.

Click <**OK**> to accept your selection.

You must save the parameter file after the export setup !

8.8.4 Export on demand

The following dialog supports manual export of program data. The data to be exported, the format as well as the export destination depend on the selected driver and its configuration. The actual data export is carried out by an export driver if you click on the <**Export**> button after a measurement.
Open the parameter file you need.
Select **File | Export** to open the **File Export** dialog box.



File Export dialog box with open driver list

Active Export Driver Select the export driver you want to use for data export. Click on the arrow button to open the list showing the available drivers and select the driver you want. ***Please keep in mind*** that you have set up the driver you have selected here in the menu **Installation | Driver | Export**. Otherwise, no data will be transferred!

Export Target Information **File Name**

Shows the file name of the active parameter file. An extension identifying the selected driver is appended (XLS for Excel files and TXT for ASCII files). The file name can be edited.

Directory

The target directory has been defined by the selected export driver during installation. Click the <**Browse**> button to select another target directory.

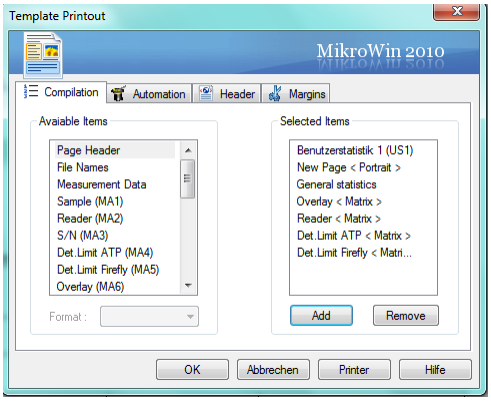
Click <**OK**> to accept your selection.

The export will be executed automatically if selected in the respective protocol file.

8.8.5 Data Print-out

Data and results can be printed automatically whenever a measurement with a parameter file has been done - ***Automatic Print-out*** has to be checked as described in the previous chapters – or on demand for the currently loaded data file.

1. Go to **Compilation** in the **Printout** section in the **Template** tab
2. Select the appropriate items by highlighting and clicking **<Add>**



3. You may check the settings and layout by selecting **Print Preview** in the **File** menu to get a preview of the print-out
4. Select **Print** in the **File** menu to start printing the data

The print-out will be executed automatically if checked when created in the respective parameter file.

8.8.6 Print-out of parameter file settings

All settings including the calculations can be printed by clicking **Template Print-out** in the **File** menu. The information will be presented as a HTML file in the web browser from where you can print the content.

9. Maintenance

9.1 Cleaning the Instrument

9.1.1 Cleaning the instrument surface

The **surface** of the instrument is protected by a washable finish. Dirty or dusty surfaces should be cleaned using a damp cloth or optical grade tissue. If necessary, use a mild detergent or diluted EtOH.

Do not use a scouring agent!

For bio-hazardous spills use an appropriate disinfectant, e.g. 5 to 10 % bleach.

9.1.2 Cleaning the inside of the instrument

The inside of the instrument does not need to be cleaned regularly. Only in case liquid spillage it may be necessary to clean the inside.

Do not open the instrument by yourself! Call a Berthold Technologies technical support person.

Before opening the instrument, turn it off and disconnect it from power supply!

Open the screws on the instrument cover to clean the instrument inside. Then detach the cover by moving or lifting it carefully.

Always keep the sample holders and the entire inside of the instrument clean. Wipe off any dirt using a damp cloth or optical grade tissue. Use cotton buds for corners. Remove dirt quickly so it does not get fry and may not have any adverse effect on moving parts.

9.2 Cleaning Tubing

Injector tubing has to be washed

- ❒ before starting work
- ❒ before changing reagents
- ❒ at the end of each work session before turning off the instrument
- ❒ after longer periods of inactivity

Berthold Technologies´ cleaning solution CLEANIT Standard* (product code 45218) *is an efficient and proven cleaning solution for most of the common reagents in use. It is recommended to use this solution at least once a weak to ensure a long lifetime of the injectors!

For daily cleaning you may use solutions recommended by the kit manufacturer.

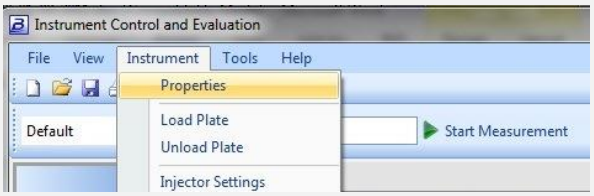
Other recommended cleaning reagents are
- *deionised water*
- *diluted alcohol: 70 % Ethanol, Propanol*
- *2 - 5 % hypochlorite solution ("bleach")*
- *0.5 – 1 M Chloric acid (HCl)*
- *0.5 – 1 M Sodium hydroxide (NaOH)*
- *0.1 % SDS*
- *Non-foaming detergent (up to 10 %)*

Some of these reagents may be hazardous. Please refer to the respective safety instructions (e.g. R and S codes) of the supplier.

Injector tubing has to be primed

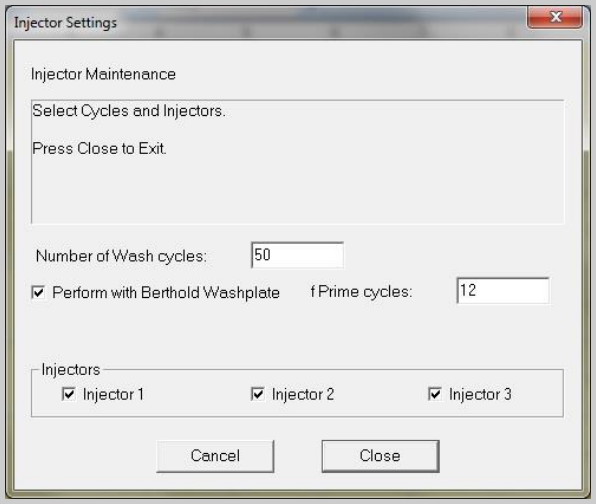
❒ prior to each measurement using the respective reagents.

1. Load the Wash Plate (or another 96 well plate)

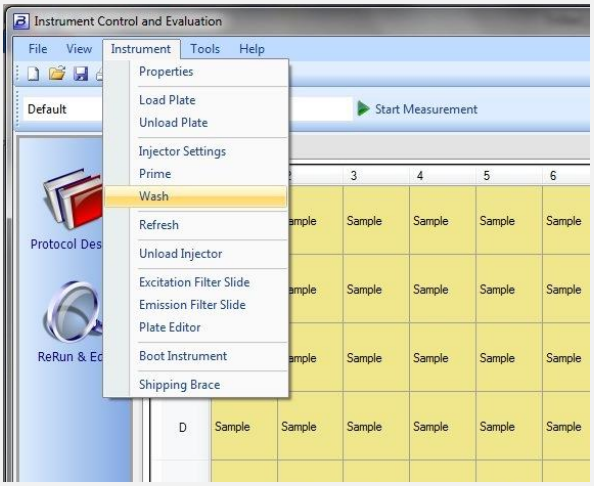


2. Click **Injector Settings** in the **Instrument** menu
3. Define the **default number of wash cycles** – 50 is recommended
4. check the **use of the Berthold Technologies Wash plate** (when available)

   Note: without the wash plate a maximum of 24 cycles is possible



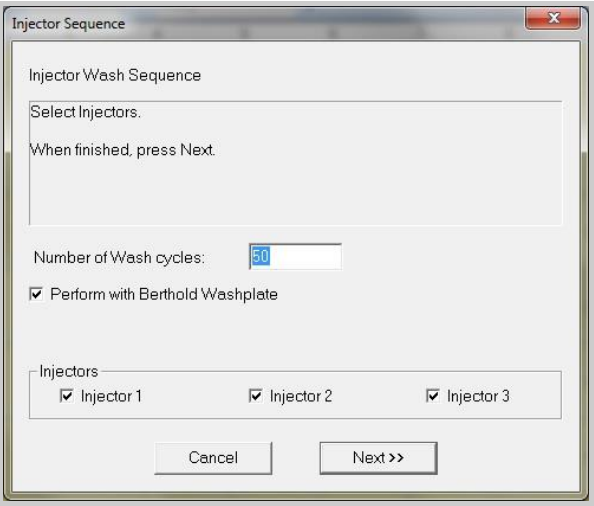
5. Click **Wash** in the **Instrument** menu



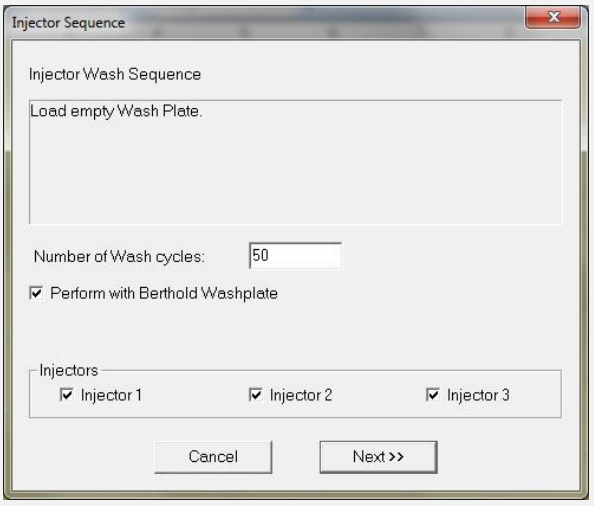
6. Define the number of **Wash Cycles**

   Make sure the total Wash volume does not exceed the volume of the plate being used!

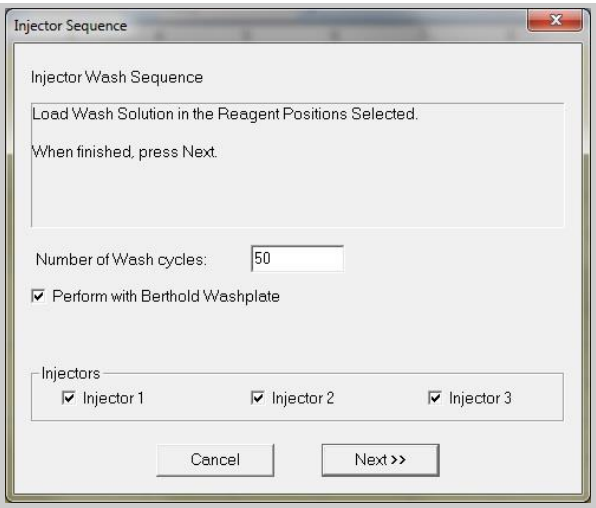
7. Select the respective **injector(s)**
8. Click **<Next>**



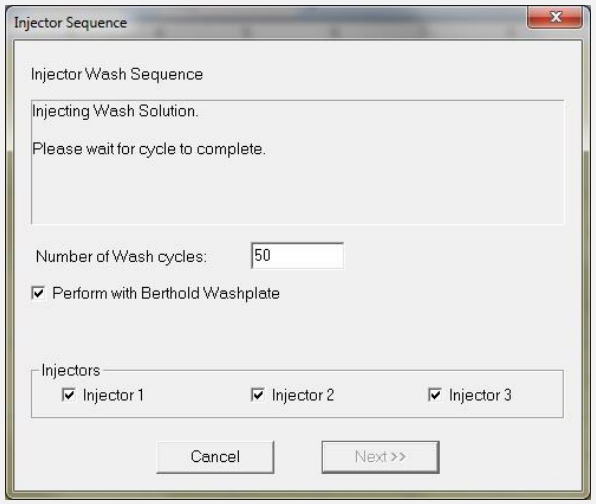
9. Load a **Wash plate**
10. Click **<Next>**



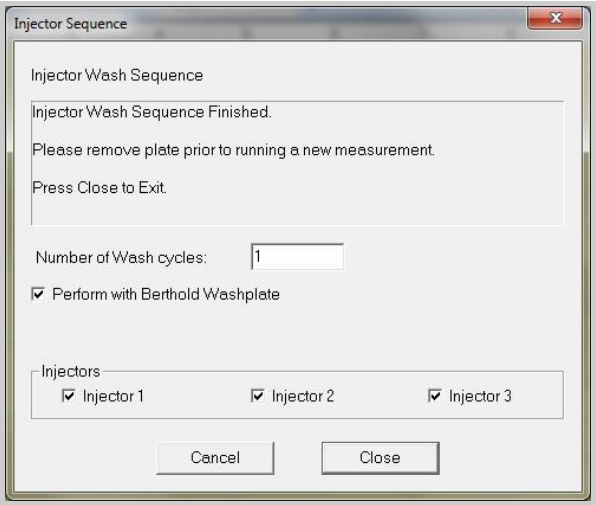
11. Attach the reservoir containing the appropriate-**Wash Solution** (see above)
12. Click **<Next>**



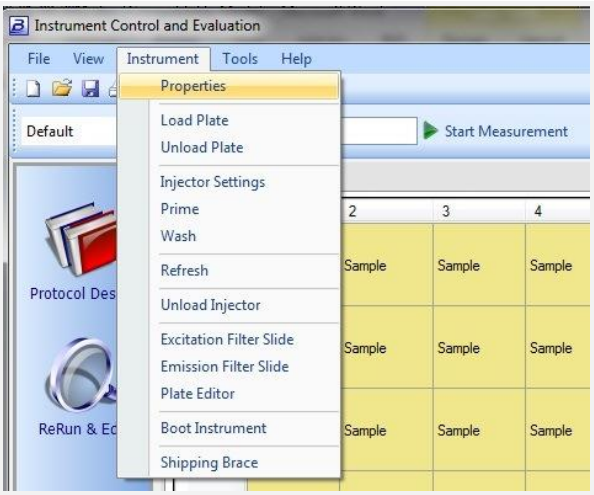
13. Wait until the wash cycles are completed



14. Click **<Close>**



15. **Remove Wash plate** by clicking **Unload Plate** in the Instrument menu



Note: It is recommended to leave deionised water in the injection lines during idle periods of hours up to a few days.

Only in case the instrument stays idle for multiple days up to weeks it is recommended to empty the lines by starting the Wash procedure without a wash solution.

9.3 Priming Tubing

9.3.1 Priming before Measurement

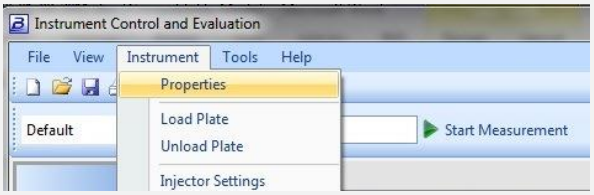
Injection lines have to be primed (filled) prior to measurements which require the use of injectors for reagent addition.

Note: It is strongly recommended to perform the priming with deionized water first and leaving the lines filled with deionized water before priming with reagents.

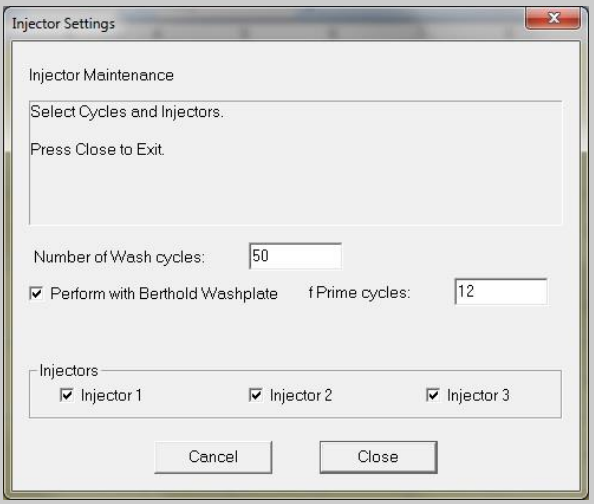
This procedure avoids reagents aerosol splashes at the injector tips and thus contamination of the instrument.

Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

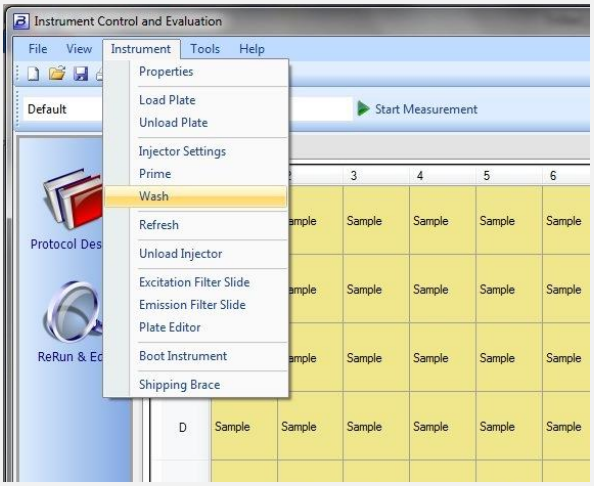
1. Load the Wash Plate (or another 96 well plate)



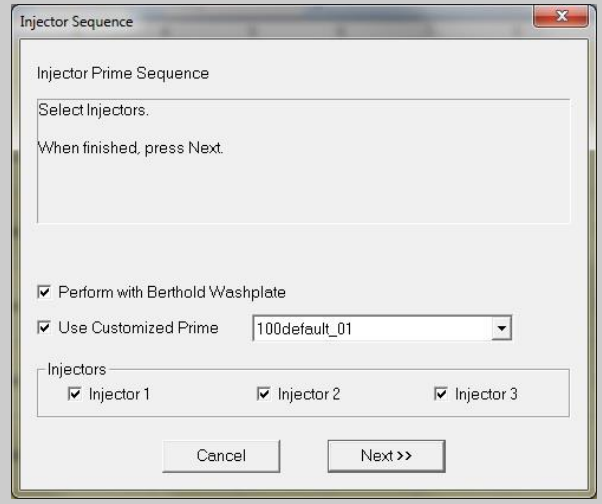
2. Click **Injector Settings** in the **Instrument** menu
3. Define the **default number of prime cycles** – 12 is recommended – which will be used for the default priming and check the **use of the Berthold Technologies Washplate**

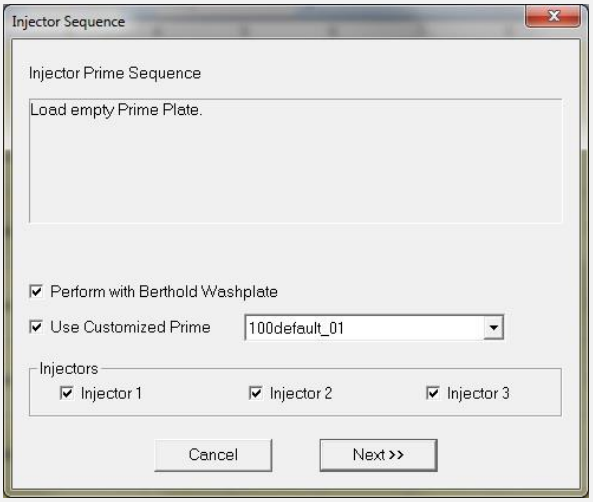
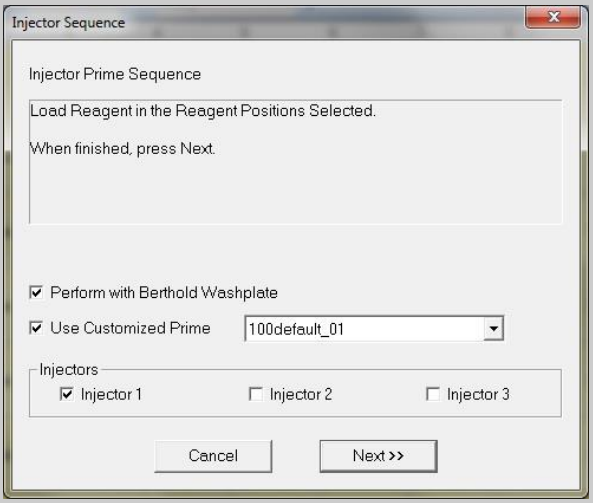
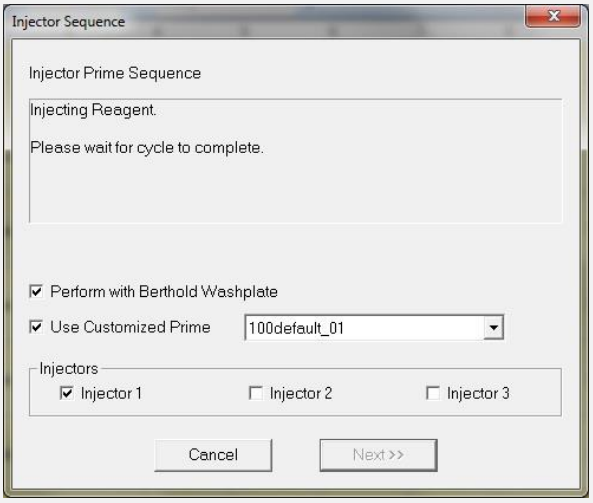


4. Click **Prime** in the **Instrument** menu

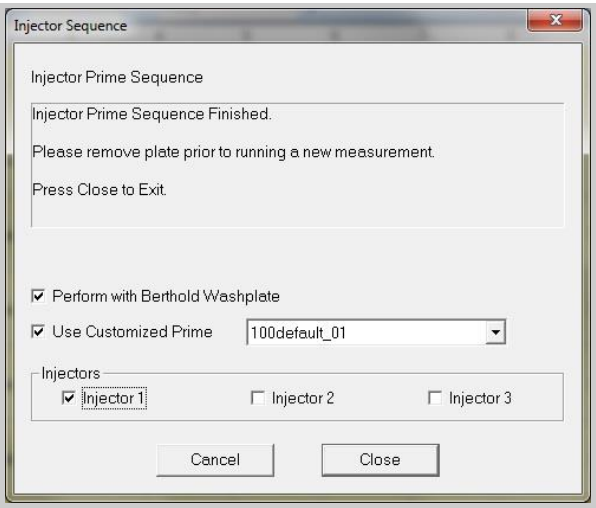


5. Check Berthold **Washplate** (when available)
6. Select the prime mode
   a. Check **Use Customized Prime** to select a user defined method (see next chapter)
   b. Uncheck Use Customized Prime to use the plain prime mode (12 straight injection cycles)
7. Select the respective **injector(s)**
8. Click **<Next>**

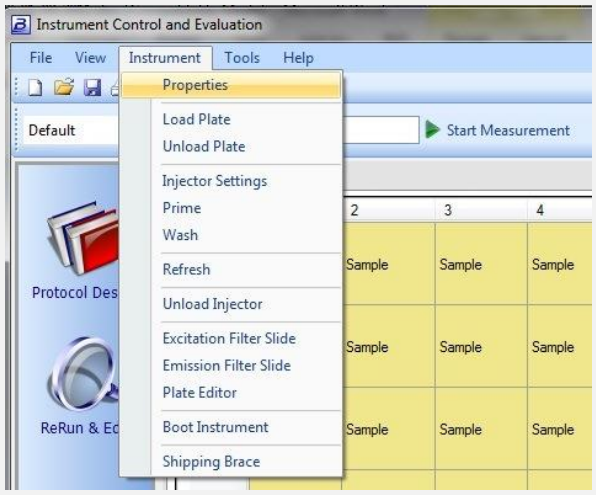


9. Load a prime plate / wash plate 10. Click **<Next>**	
11. Attach the reservoir(s) containing the appropriate **Assay Reagents** (or deionized water; see above) 12. Click **<Next>**	
13. Wait for the Prime procedure to be finished for one injector	

14. Click **<Close>**



15. **Remove prime / wash plate** by clicking **Unload Plate** in the Instrument menu



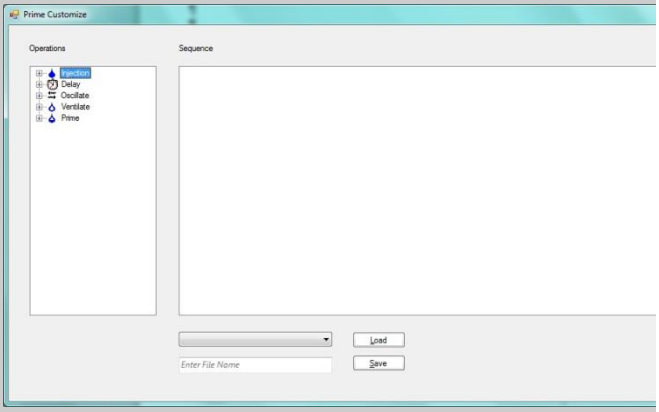
16. The instrument is now ready for use

9.3.2 Customizing the Priming Sequence

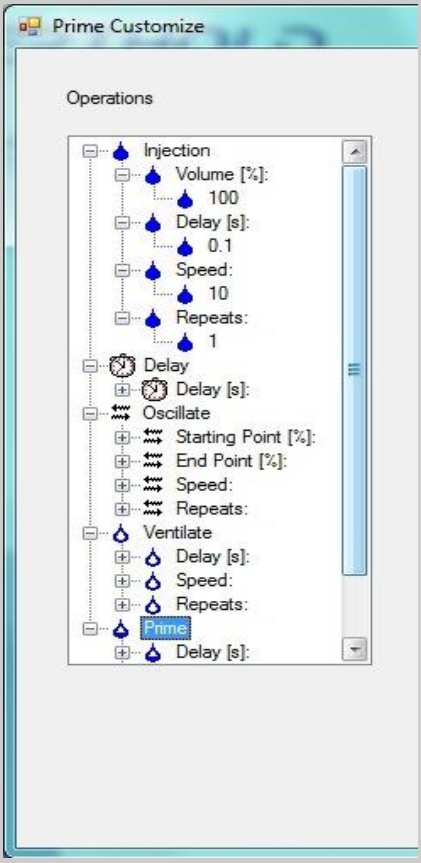
Some reagents (e.g. high viscosity) or solutions (e.g. cells) require special priming procedures which can be defined individually.

1. Click **Customized Prime Settings** in the **Instrument** menu

2. The Prime Customize dialog will be displayed



clicking on ⊞ in the Sequence window will expand the respective folders and display the settings



3. The respective operation can be selected for the prime sequence by dragging it from the left column to the right column (**Sequence**)

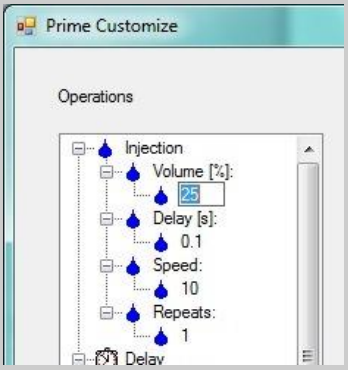
to change the sequence the arrow buttons can be used

to remove an operation the button  can be used

The operations and their settings:

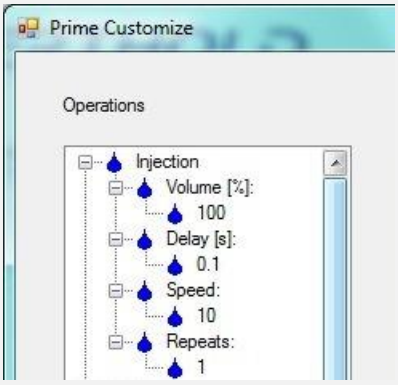
To change the settings

- expand the operation
- expand the setting
- click onto the number
- click onto the number a 2nd time
- type the appropriate number
- confirm with the **ENTER** key



a. **Injection**
the injector is filled with the max. injection volume from the reagent reservoir and injects the set volume

Volume	percentage of the max. inj. vol.
Delay	delay before the operation in sec
Speed	1 … 10
Repeats	number of repeats

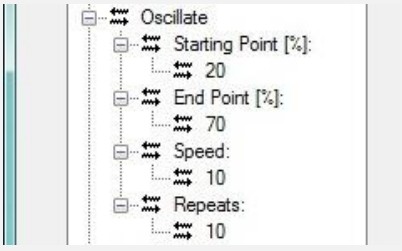


b. **Delay**
a delay time that elapses between operations, e.g. to mimic the injection timing of the assay (this can be important with a cell suspension)

Delay	elapsing time in sec

c. **Oscillate**
the injector is (partly) filled and oscillates between the set positions (back into the reagent reservoir)

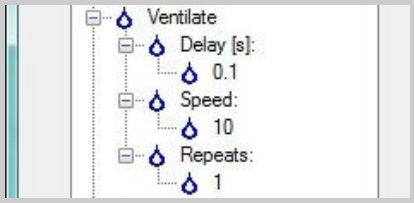
Start. Point	percentage of the max. inj. vol.
End Point	percentage of the max. inj. vol.



Speed	1 … 10
Repeats	number of repeats

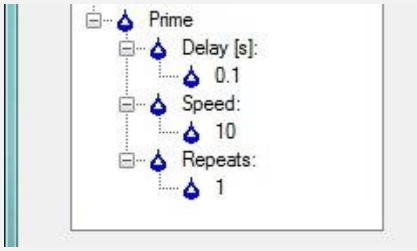
d. **Ventilate**
the injector is completely filled (beyond the max. injection volume) from the reagent reservoir and injects the total volume of the bellow

Delay	delay before the operation in sec
Speed	1 … 10
Repeats	number of repeats



e. **Prime**
the injector is filled with the max. injection volume from the reagent reservoir and injects the full volume

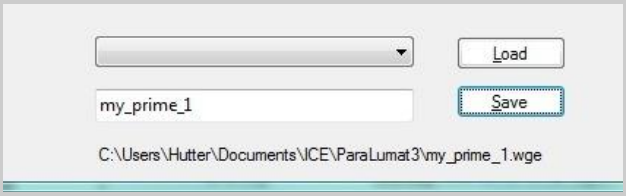
Delay	delay before the operation in sec
Speed	1 … 10
Repeats	number of repeats

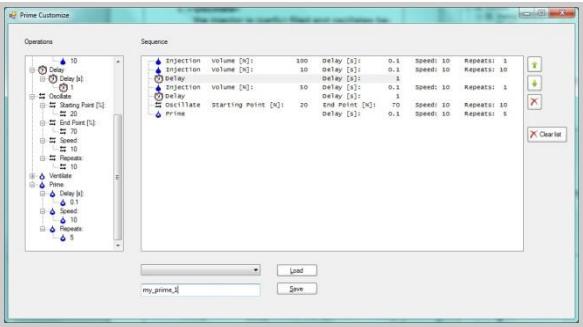


4. After the sequence is completed enter a **name** for this priming sequence and click **<Save>**

   the file will get the extension .wpe

   the respective directory will be displayed





5. Close the dialog by clicking

9.4 Emptying Tubing

This operation can be used to empty the injection lines after the measurement and re-collect valuable reagents in the reagent reservoirs.

Note: Make sure the reagent reservoir are connected to the injection tubings !

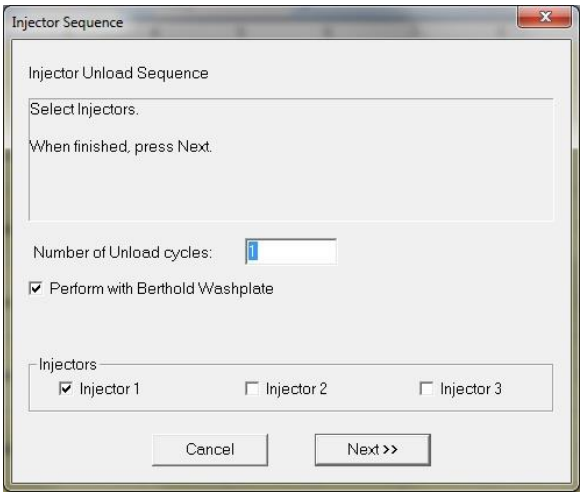
1. Click **Unload Injector** in the **Instrument** menu

2. Define the **Number of Unload cycles**

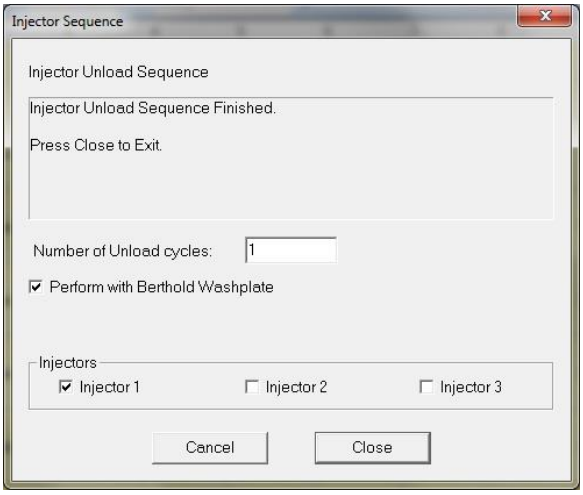
   each cycle is equivalent to the max. injection volume of the injector installed

   a minimum of 10 is recommended

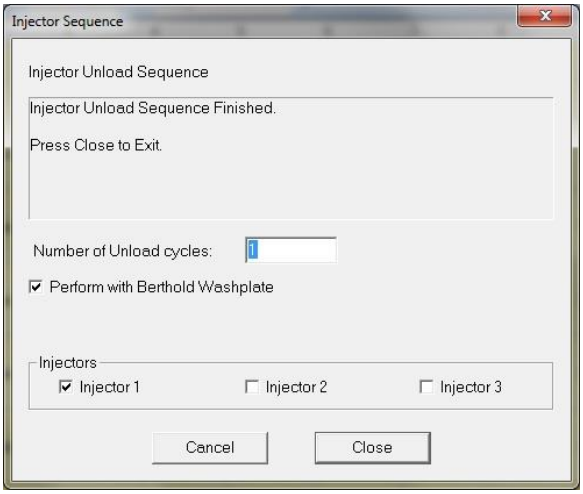
3. Define whether **Injector 1** or **Injector 2** or **Injector 3** or any combination are to be emptied
4. Click **<Next>**



5. Wait for the unload operation to be finished



6. Click **<Close>**



9.5 Preparations for transport

The **following safety provisions have to be taken** to transport or ship the instrument:

- Remove the microplate from the instrument
- Turn instrument off and **disconnect it from mains**
- Make sure the instrument is decontaminated properly before removing it from the laboratory and fill in the decontamination form
- Click <**Shipping Brace**> in the **Instrument** menu
- Turn instrument off and disconnect it from mains
- Insert transport safety device(s)

- For shipping you **must** use the **original transportation case**
- Encase the instrument with the styrofoam parts
- **Tape shipping carton tightly**
- Have a filled in **Declaration on Decontamination** accompany the instrument when shipping back to Berthold Technologies or one of its representatives

10. Trouble shooting

Symptom	*Possible cause*	*Solution*
LED flashes red accompanied by 2 beeps	CAN module not correctly installed	1) switch instrument off and on again 2) call service
LED stays orange	Cable between instrument and computer is not connected Wrong COM assigned	1) attach cable properly 2) use service software and run “Scan COM ports” command
Instrument does not respond to software commands (status “Timeout Error”)	Cable between instrument and computer is not connected Wrong COM assigned	1) attach cable properly 2) use service software and run “Scan COM ports” command
LED stays dark	Instrument not switched on Mains not plugged in mains supply deactivated mains plug defective	1) switch instrument on 2) plug in mains 3) check with local house electrician 4) call service
Lower signal than expected	Pipetting/preparation error substrate consumed	1) verify by checking replicate and other samples / controls / standards and prepare faulty sample again 2) prepare new plate and read immediately after adding substrate
Signal not above background readings	No sample No reagents added	1) check sample preparation 2) add reagents
No signal at all	Faulty PMT	Call service
Plate is not moved to measurement position	Plate not correctly inserted Wrong frame Plate too high	1) insert plate correctly 2) change frame 3) use another plate with a total max. height of 16 or 21 mm respectively

Error message no plate	No plate Wrong frame	1) insert plate 2) insert black frame for 15 mm plates
High background signal	Reagents not prepared properly Reagents contaminated Plate contaminated	3) prepare reagents properly 4) prepare fresh reagents 5) use another clean plate 6) call service
Standard curve cannot be calculated	Standards pipetted in wrong order	1) prepare new plate with correct layout of standards 2) use the edit function in the standard curve tab
Excel Files cannot be opened	Excel is not installed	Install Excel
Adobe PDF files cannot be opened	Adobe Acrobat Reader is not installed	Install Adobe Acrobat

11. Technical Data

Mains Supply (for external power adapter)	100 – 240 VAC ±10% 50 / 60 Hz Class I
Operating voltage	24 VDC ± 5%
Power consumption	140 VA
Certifications	CE, UL, CSA
Safety standards	IEC 61010-1:2010, IEC 61010-2-010:2014, IEC 61010-2-081:2015, IEC 60825-1:2007, IEC 60825-1:2014, EN 61326-1:2013 EN 61000-3-2:2006 + A1:2009 + A2:2009, EN 61000-3-3:2008
Installation category	II
Temperature range	storage: 0° - 40°C operation: 15° - 35°C
Humidity	10 – 80%, not condensing maximum relative humidity of 80 % for temperatures up to 31 °C decreasing linearly to 50 % relative humidity up to 40 °C
Altitude	Max. 2000 m (above sea level)
Pollution degree	2
Dimensions	391 x 470 x 344 mm (W x D x H) 391 x 470 x 389 mm (W x D x H) (depending on variant)
Weight	Up to 26,6 kg (depending on variant)
Plate formats	6 to 384 well, solid and strip Dimensions 128 x 86 mm (L x W), height 14.0 – 21.0 mm (adapters necessary Petri dishes 35 and 60 mm Eppendorf µPlate G 0.5 Standard cuvettes (with cap)
Measurement technology	Luminescence Fluorescence Absorbance Time-Resolved Fluorescence
Operation modes	Integral measurement 0.05 – 600 seconds (single and multiple endpoint) Kinetics measurement (total length up to 24 h) Repeated measurement (total length up to 7 days) Plate repeats (up to 50,000) Scanning (up to 10,000 single data points) Spectral Scanning Dispensing with 4 independent variable injectors Shaking Delay (up to 600 second) Unload
Excitation source	Xenon flash lamp, 10 W, 200 to 1000 nm
Detector	Photomultiplier operated in single or dual mode Photodiode

<table>
<tr><td>Monochromator</td><td>f number: 2.7 (high light transmission)
variable bandwidth: 4 - 22 nm
increment: 1 nm
blocking: 10^{-6}</td></tr>
<tr><td>Excitation filters</td><td>Ø 15 mm or 12.7 mm with adapter; 25 mm</td></tr>
<tr><td>Emission filters</td><td>Ø 25.4 mm</td></tr>
<tr><td>Sensitivity</td><td>Luminescence:
ATP: <6 amol/well (96)
Fluorescence:
FITC: <200 amol/well (384)
Absorbance:
Accuracy better 1 %, precision better 0.3 % (96 well, 2.5 OD)
Time-Resolved Fluorescence:
Eu: <5 amol/well (96)</td></tr>
<tr><td>Dynamic Range</td><td>6 orders of magnitude (photon counter)
4 OD (photodiode)</td></tr>
<tr><td>Crosstalk</td><td>10^{-6} (black plates)</td></tr>
<tr><td>Injector</td><td>up to 3 injectors, JET injection technology
variable volumes: 10 – 100 µL (option: 25 – 300 µL
speed 200 – 440 µL/sec
accuracy ø 99 %, precision ø 99 %
injections into microplates with up to 384 wells</td></tr>
<tr><td>Temperature control</td><td>Optional: +5°C above room temperature to 45°C</td></tr>
<tr><td>Shaking</td><td>3 modes, variable amplitude and speed
Linear & orbital mode
<table>
<tr><td></td><td colspan="3">amplitude</td></tr>
<tr><td>speed</td><td>0.1mm</td><td>2.5mm</td><td>5.0mm</td></tr>
<tr><td>low</td><td>438</td><td>42</td><td>22</td></tr>
<tr><td>normal</td><td>882</td><td>90</td><td>44</td></tr>
<tr><td>fast</td><td>1758</td><td>174</td><td>88</td></tr>
</table>
Double orbital mode
<table>
<tr><td></td><td colspan="3">amplitude</td></tr>
<tr><td>speed</td><td>0.1mm</td><td>2.5mm</td><td>5.0mm</td></tr>
<tr><td>low</td><td>219</td><td>21</td><td>11</td></tr>
<tr><td>normal</td><td>441</td><td>45</td><td>22</td></tr>
<tr><td>fast</td><td>879</td><td>87</td><td>44</td></tr>
</table></td></tr>
<tr><td>Interface(s)</td><td>USB</td></tr>
<tr><td>Operating system</td><td>Win Win 7, Win 8, Win 8.1, Win 10</td></tr>
<tr><td>PC requirements</td><td>Pentium, 1 GHz (or better), CD ROM drive, 1 free USB port</td></tr>
<tr><td>Software</td><td>wizard support for parameter entries (ICE only)
input of plate format
selection of wells
raw data assays (reporter genes, caspases, etc)
dual raw data assays (e.g. dual reporter genes)
kinetic
repeated
scanning
spectral scanning
ratio calculation or subtraction</td></tr>
</table>

	data export: EXCEL

12. Appendix

a. Customer Reply Form

Send Customer Reply Form to:

Berthold Technologies GmbH & Co KG
Technical Support
Calmbacher Str. 22
75323 Bad Wildbad
Germany
Phone: +49 7081 177 114
Fax: +49 7081 177 301
Email: service@berthold.com

or **your local representative**.

A blank Customer Reply Form can be found overleaf.

Customer Reply Form

Date: Customer no.:

Name:

Company:

Department:

Address:

Address:

Phone: Fax:

Email:

Instrument:

ID no.:

Serial no.:

Embedded software version:

Instrument driver software version:

Accessory instruments:

PC Software: PC software version:

Windows version:

Computer type: CPU type:

Other installed software:

Time when problem occurred (Windows clock):

Error message(s):

Description of the problem:

b. Decontamination Remarks

The user must make sure

- proper decontamination is performed when hazardous substances are spilled on or inside the equipment
- no decontamination or cleaning agents are used, which may cause a hazard due to a reaction with parts of the machine or the materials contained in it
- Berthold Technologies or its representative will be contacted if there is any doubt regarding the compatibility of decontamination or cleaning agents with parts of the machine or the materials contained in it
- Decontamination should be performed by authorized and trained personnel only wearing appropriate protective devices (e.g. gloves, eye-wear, mask) and in an appropriate and safe environment (e.g. safety cabinet).
- Suitable disinfection agents may be
  - TriGene
  - Chloros/bleach
- Conduct decontamination/disinfection according to the solution manufacturer´s instructions
- Disconnect the instrument from power and from computer before conducting the procedures
- The decontamination/disinfection solution can negatively impact the performance of the instrument.

c. Confirmation on Decontamination Form

Confirmation on Decontamination

If you return an instrument to BERTHOLD TECHNOLOGIES for servicing purposes which is not properly decontaminated, there will be a health risk for BERTHOLD TECHNOLOGIES employees. We therefore need your confirmation that the instrument was decontaminated and cleaned properly before shipping. If the form below is not filled in accordingly and completely, we are forced to reject the instrument. Please understand that this is intended to protect our employees from any hazards.

Please put one copy into the shipping box and a duplicate into an envelope attached to the outside.

(**Please use capital letters!**)

instrument / component: _____ serial no.: _____

instrument or component has come into contact with:

[ ] radioactive substances — means of decontamination applied:
Isotope(s): _____ _____
_____ _____

[ ] chemical reagents — means of decontamination applied:
specify: _____ _____
R and S rules: _____ _____

[ ] biological material — means of decontamination applied:
specifiy: _____ _____
_____ _____

[ ] contagious agents — means of decontamination applied:
specify: _____ _____
_____ _____

indicate security level of the laboratory the instrument has been used in

[ ] S1 [ ] S2 [ ] S3 [ ] S4

[ ] I hereby confirm that the instrument or component specified above was not contaminated with any of the above mentioned substances / reagents / agents

[ ] I hereby confirm that the instrument or component specified above was decontaminated / cleansed using the appropriate method

date: _____ signature: _____

name: _____ address: _____
_____ _____
title: _____ _____
phone: _____ _____
fax: _____

7-40.12 – 31531/03

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