Betriebsanleitung
Operating Manual

LB 514
FlowStar²

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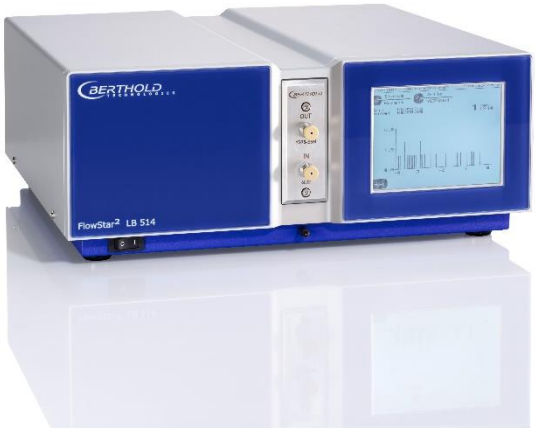

Diese Geräte sind nicht für den Betrieb in explosionsgefährdeten Bereichen bestimmt!
Die gelieferten Geräte dürfen nur vom Service der Firma Berthold oder durch von der Firma Berthold autorisierte Techniker instand gesetzt werden!

These units are not designed for use in hazardous areas.
The units supplied should not be repaired by anyone other than Berthold service engineers or technicians authorized by Berthold.

Im Störungsfall wenden sie sich bitte an unseren zentralen Kundendienst.

In case of operation trouble, please address to our central service department.

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1. Introduction

1.1 Contents

1.2 Typographical Conventions

We will use the following typographical conventions:

Caution! Danger!

➔ **Important information!**

	Example
Menus and **options** in the **FlowStar software** are printed in bold type-face	**Measurement**
The **selection of an option on a menu** is written as a sequence of commands, separated by a vertical line	**Device | Configuration**
Display pages and **parameters** are also printed in bold type-face	**Edit step**
Function keys are printed in bold type-face inside angular brackets	<**Start**>, <**Stop**>
Enumerations are highlighted by	●
Action steps are identified by	❑

1.3 Safety Instructions

Special instructions and precautions

Caution! This sign alerts you to important operating procedures with a potential danger of damaging the equipment and endangering your safety on disobeying. Refer to the user and instrument manuals for precautionary instructions.

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 do always act 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 below 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 instrument has been manufactured in accordance with the safety requirements for electronic and medical measuring systems. If the law lays down regulations on the installation and/or operation of sample measuring system, 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. Using the instrument in other manner than described in the manual can result in damage of the instrument, other system components and can also cause user injuries.

The instruments are tested by the manufacturer and 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.
- Please observe the legal requirements concerning work with radioactive material! Without special permit the instrument must always be used with radioactivity below the permitted limit. The manufacturer cannot guarantee a maximum radiation of 1µSv/h in a 100mm distance. When using high activities the user has to make sure this limit is not exceeded.
- Operate the instrument inside a collecting pan to avoid uncontrolled contamination in case of leakage.
  The instrument is meant to be used with solvents commonly used in chromatography.
- The installation category is II.
- 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.
- Use the instrument only for the designated application.
- The instrument is designed for indoor use only.
- The instrument may not be operated at altitudes above 2000 m above sea level.

- The instrument is designed to be operated within a temperature range of 15 to 30 °C.
- The instrument is designed to be operated at a maximum relative humidity of 80 % for temperatures up to 31 °C decreasing linearly to 50 % relative humidity up to 40 °C.
- BERTHOLD TECHNOLOGIES assumes no liability for any damages, including those to third parties, caused by improper use or handling of the instrument.
- 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 fluctuations must not exceed +/- 10 % of the nominal voltage. Maximum voltage to be applied is 264 VAC.
- The instrument 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.
- The instruments are designed according to the IEC 61010-1 or EN 610 10-1 regulations for electrical measuring systems.

- To disconnect the instrument from power the appliance coupler has to be removed from the mains. The mains inlet of the instrument is located at the rear panel (see 7.2).
- 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.
- Pull the power cord to disconnect instrument from power supply.
- Turn instrument off before pulling the power cord.
- 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 and remove the flow cell Clean the measuring chamber or have it cleaned by an authorized service center.
- Do not use any flammable or explosive solutions or liquids whose mixture is flammable or explosive.
- The instrument is not intended to be used with biohazardous samples.
- The operator is responsible for the use of reagents.
- 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.
- Pollution degree is 2.

- This instrument must not be used in areas with potentially explosive atmosphere.
- The instrument may only be used in rooms with a max. pollution degree of 2.
- The instrument and the flow cells are designed for flow rates of max. 10ml/min. If you intend to use higher flow rates, please contact the manufacturer.
- The total system pressure must not exceed 200kPa l.
- The 12V terminals at the rear connectors do not comply with the requirements of limited-energy circuits.
- The instrument is part of a measuring system with external HPLC-Controller, PC and / or other equipment. Hazards resulting from application caused by supplied measuring substances and evaluation of the measured values by external analyses must be considered in end system / application.

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. Use the instruments only for the designated application.

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1.4 Sicherheitshinweise

Die vorliegende Bedienungsanweisung enthält Informationen und Warnungen, die vom Benutzer befolgt werden müssen, um einen sicheren Betrieb der Geräte zu ermöglichen.

Dieses Zeichen weist den Benutzer auf wichtige Punkte hin, deren Beachtung unerlässlich ist.

Die folgenden Sicherheitshinweise sind sowohl vor der Inbetriebnahme als auch während des Betriebs des Gerätes unbedingt zu beachten. Vor Inbetriebnahme des Gerätes ist es zwingend erforderlich, die Bedienungsanleitung zu lesen, da ansonsten die Sicherheit des Gerätes und des Benutzers nicht gewährleistet wird.

Das Gerät wurde in Übereinstimmung mit den Sicherheitsanforderungen für elektronische und medizinische Messgeräte hergestellt. Bestehen für die Errichtung und/oder den Betrieb von Probenmessgeräten gesetzlich vorgeschriebene Regelungen, so ist es die Aufgabe 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. Nicht bestimmungsgemäßer Gebrauch im Gesamtsystem kann Beschädigungen am Gerät, anderen Systemkomponenten oder zu Verletzungen des Benutzer führen.

Die Geräte sind werkgeprüft und wurden in betriebssicherem Zustand ausgeliefert.

- ❑ Die Geräte dürfen nur von autorisierten Personen in Betrieb genommen und nur von eingewiesenem Personal bedient werden. Alle Benutzer, die mit den Geräten arbeiten, müssen zuerst diese Bedienungsanleitung lesen.

- ❑ Bitte beachten sie die örtlichen Bestimmungen zum Umgang mit radioaktiven Stoffen. Ohne Umgangsgenehmigung dürfen nur Aktivitäten unterhalb der Freigrenze verwendet werden. Der Hersteller kann nicht für die Einhaltung des Dosisgrenzwertes von 1µSv/h in 100mm garantieren. Bei Verwendung hoher Aktivitäten ist der Betreiber für die Einhaltung des Grenzwertes verantwortlich.
  Betreiben sie das Gerät in einer geeigneten Auffangschale und schließen sie den Messkammerablauf an ein geeignetes Sammelgefäß an um unkontrollierte Kontamination im Falle eines Lecks zu vermeiden.
- ❑ Das Gerät ist für die Benutzung aller gängigen Eluenten in der Chromatographie vorgesehen.
- ❑ Installationskategortie ist II.
- ❑ Die Geräte dürfen nur von dafür geschultem Personal betrieben werden. Es wird allen Anwendern empfohlen, diese Bedienungsanleitung vor Benutzung zu lesen.
- ❑ Transportsicherungen vor dem Einschalten entfernen.
- ❑ Die Geräte dürfen nur für den vorgesehenen Zweck eingesetzt werden.

- ❑ Berthold Technologies übernimmt keinerlei Gewährleistung, auch für Schäden gegenüber Dritten, die durch unsachgemäße Handhabung der Geräte hervorgerufen werden.
- ❑ Die Geräte dürfen nur innerhalb geschossenen Räumen betrieben werden.

- ❑ Die Geräte dürfen nicht in Höhen von mehr als 2000 m über dem Meeresspiegel betreiben werden.
- ❑ Die Geräte sind dafür ausgelegt, innerhalb des Temperaturbereiches von 15 bis 40 °C betrieben zu werden.
- ❑ Die Geräte sind dafür ausgelegt, bei einer maximalen relativen Luftfeuchte von 80 % (bis zu 31 °C) betrieben zu werden, die linear auf 50 % (bei 40 °C) absinkt.
- ❑ Die Stromversorgung darf nicht mehr als ±10 % des Nominalwertes aufweisen. Maximal sind 264 V Wechselstrom erlaubt.
- ❑ Es liegt im Verantwortungsbereich des Anwenders, dass die Geräte nach den lokalen elektrischen Vorschriften installiert werden.

- ❑ Die Geräte 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 Anschusses.
- ❑ Die Geräte entsprechen den Vorschriften der IEC 1010-1 und EN 61010-1 für elektrische Messgeräte.
- ❑ Nicht öffnen, wenn das Gerät in Betrieb ist.
- ❑ Service- und Reparaturarbeiten dürfen nur von Fachleuten ausgeführt werden.
- ❑ Es dürfen nur die im Handbuch beschriebenen Wartungsarbeiten vom Anwender ausgeführt werden.
- ❑ Bei Wartungsarbeiten dürfen nur die angegebenen Teile verwendet werden.
- ❑ Um das Gerät vollkommen vom Netz zu trennen, kann das Netzkabel gezogen werden. Der Netzeingang befindet sich auf der Rückseite (siehe 7.2)
- ❑ Gerät ausschalten, bevor der Stecker gezogen wird.
- ❑ Alle gelieferten Geräte und Zusatzgeräte sind geerdet ans Netz anzuschließen. Schutzkontaktstecker verwenden!
- ❑ Stellen Sie das Gerät so auf, dass Sie es leicht ein- und ausschalten können.
- ❑ Bei Beeinträchtigung der Betriebssicherheit sind die Geräte abzuschalten und vom Netz zu trennen.
- ❑ Ist Flüssigkeit in das Innere des Gerätes gelangt, Netzstecker ziehen. Die Messzelle entfernen und Messkammer reinigen bzw. durch eine autorisierte Servicestelle reinigen lassen.

- ❑ Es dürfen keine entzündlichen oder explosiven Flüssigkeiten oder keine Flüssigkeiten, deren Mischung entzündlich oder explosiv ist, verwendet werden.
- ❑ Das Gerät ist nicht für den Einsatz von biologisch gefährdenden Substanzen vorgesehen.
- ❑ Beachten Sie alle gesetzlichen Vorschriften für den Umgang mit biologischem Abfall, mit Reagenzien und Patientenproben.

- ❑ Die Anwendung der Reagenzien liegt im alleinigen Verantwortungsbereich des Benutzers.

- Das Gerät sollte nur in der eigenen Verpackung transportiert werden. Beim Transport ist darauf zu achten, dass alle Transportsicherungen eingesetzt werden (z.B. die Sicherung für den Plattenträger).
- Zum Reinigen des Gerätes bitte den entsprechenden Teil dieser Bedienungsanleitungen beachten.
- Ordungsgemäße Funktionalität kann nur bei Verwendung der Originalersatzteile garantiert werden.
- Verschmutzungsgrad ist 2.
- Das Gerät darf nicht in explosionsgefährdeten Bereichen verwendet werden.
- Das Gerät darf nur in Räumlickeiten mit einem maximalen Verschmutzungsgrad von 2 betrieben werden.
- Die Messzellen und das Gerät sind für eine Flussrate von max. 10ml/min vorgesehen. Sollten sie beabsichtigen höhere Flussraten einzusetzen, kontaktieren sie bitte den Hersteller.
- Die 12V Anschlüsse an der Geräterückwand entsprechen nicht den Anforderungen an sog. „limited-energy circuits".
- Das Gerät ist Teil eines HPLC-Gesamtsystems mit Steuermodul, PC und /oder anderen Komnponenten. Gefahren die aus der Anwendung der Proben hervorgehen sowie die Auswertung der Messwerte auf externen System müssen im Endsystem bzw. der Anwendung in Betracht gezogen werden.

Für die Sicherheit des Benutzers und die Funktionsfähigkeit der Geräte sind die vom Hersteller empfohlenen Überprüfungen und Wartungsmaßnahmen durchzuführen. Alle über die Betriebsanleitung hinausgehenden Wartungs- und Instandhaltungsmaßnahmen dürfen nur von autorisierten Technikern ausgeführt werden.

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1.5 Consignes de Sécurité

Attention! Ce symbole d'alarme, vous avertit de prêter attention aux consignes opératoires. En effet si vous ne suivez pas ces instructions, il peu y avoir un risque d'endommagement du matériel et également vous faire ecourir des risques pour votre propre sécurité. Il est impératif de respecter les instructions du mode d'emploi et de les respecter.

Ce mode d'emploi contient des informations et avertissements qui doivent être suivis par l'utilisateur afin de garantir un fonctionnement sûr des instruments.

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, autrement ni le fonctionnement correct de l'appareil ni la sécurité de l'utilisateur peuvent être garantis. Ne pas suivre ces instructions de service peut invalider la garantie.

Le appareil a été fabriqué conformément aux prescriptions de sécurité en vigueur pour les appareils de mesure électroniques et médicaux. Si l'installation et/ou l'utilisation des appareils de mesure de prélèvements-échantillons sont/est soumise(s) à des réglementations prescrites par la loi, il appartient à l'utilisateur de les respecter.

Le constructeur a fait tout 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.

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

- ❑ Veuillez respecter la réglementation en vigueur sur les matières radioactives. Sans autorisation seules des activités en-dessous de la limite d'exemption peuvent être utilisées. Posez l'appareil dans un bac de rétention et raccordez le drain de la chambre de mesure à un récipient pour déchets liquides enfin d'éviter une contamination en cas de fuite.

- ❑ Les appareils doivent être mis en service et utilisés strictement conformément aux recommandations du constructeur. La mise en service est réservée au personnel formé et autorisé.
- ❑ La catégorie de mise en service est de niveau II.
- ❑ 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 de service.
- ❑ Ne mettez jamais des parties de votre corps ou des objets dans l'appareil lorsque celui-ci est en fonctionnement.
- ❑ Enlevez le verrouillage de transport avant la mise sous tension de l'appareil.

- ❑ Utilisez l'instrument uniquement pour les applications désignées compatibles.
- ❑ L'appareil est destiné uniquement pour une utilisation en intérieur de bâtiments.
- ❑ L'appareil est destiné uniquement pour une utilisation à une altitude ne devant pas dépasser 2000 m au dessus du niveau de la mer.
- ❑ L'appareil est destiné uniquement pour une utilisation dans une température ambiante comprise entre 15 et 40 °C.
- ❑ L'appareil est destiné uniquement à une utilisation sous humidité relative ambiante maximum de 80%, pour des températures allant jusqu'à 31 °C et diminue linéairement jusqu'à 50% humidité relative pour une température d'ambiance allant jusqu'à 40 °C.
- ❑ BERTHOLD TECHNOLOGIES décline toute responsabilité de dommages résultant d'une utilisation non conforme à l'emploi prévu, y compris les dommages causés à des tiers.
- ❑ Les variations sur la tension du secteur ne doivent pas dépasser +/- 10% de la valeur nominale (max. 264 VAC).
- ❑ L'utilisateur porte la responsabilité de la mise en service de l'appareil selon les prescriptions électriques en vigueur.
- ❑ L'instrument est fourni avec une fiches à 3 broches dont une prise de terre. C'est une prescription de sécurité. Il est nécessaire que cette fiche puisse être branchée sur prise reliée à la terre. Dans le cas contraire, il vous faut alors en avertir un électricien afin d'installer une telle prise. Il ne faut pas négliger cette consigne de sécurité.
- ❑ Les appareils correspondent aux prescriptions de la norme C.I.E. 61010-1 ou EN 610 10-1 concernant les instruments de mesure électriques.
- ❑ Pour arrêter et débrancher l'instrument la fiche doit être retirée hors de la prise.
- ❑ Ne pas ouvrir le couvercle lors du fonctionnement de l'appareil. Arrêtez l'instrument avant.
- ❑ Les travaux d'entretien et de réparation devront être confiés exclusivement à des spécialistes dûment formés.
- ❑ Les travaux d'entretien uniquement décrits dans le manuel peuvent être effectués par l'utilisateur.
- ❑ Pour les travaux d'entretien, utiliser exclusivement les pièces mentionnées.
- ❑ Avant d'ouvrir l'appareil, couper l'alimentation en courant.
- ❑ Arrêter l'appareil avant de retirer la fiche.
- ❑ Si vous ouvrez l'appareil, les sécurités ne sont plus activées (capôt et parties de la façade de l'appareil). Faites attention aux parties mobiles. L'intérieur de l'appareil et certaines pièce peuvent atteindre des températures pouvant provoquer des brûlures si il y a contact. Appareil éteint,

des parties peuvent rester chaudes alors qu'il n'y a pas d'indication visible de température élevée.

- **Attention: Il y a un risque d'explosion si la pile n'est pas insérée correctement. Remplacer la pile uniquement par un pile du même type ou un type de remplacement recommandé par une personne autorisée. Les piles usagées sont à éliminer conformément aux instructions et prescriptions de votre pays.**
- Positionner l'appareil de manières à ce que les interrupteurs soient accessibles.
- 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 débrancher. Ouvrir l'appareil et le nettoyer ou bien le faire nettoyer par une agence de service-après vente autorisée.
- Ne pas utiliser des liquides inflammables ou explosifs ni de liquides dont le mélange est inflammable ou explosif.
- Respecter toutes les prescriptions légales concernant la manipulation des déchets biologiques, des réactifs et des prélèvements-échantillons de patients.
- **L'utilisateur assume la responsabilité exclusive de l'utilisation des réactifs.**
- Pour le nettoyage de l'instrument veuillez vous référer au paragraphe correspondant dans ce mode d'emploi.
- Le fonctionnement correcte ne peut être garantit qu'à la condition que des pièces de rechange appropriées sont utilisées.
- Degré de pollution est de niveau 2.
- Ne pas utiliser les instruments dans des pièces ou à des places ou il y a un risque d'explosion.
- L'appareil est destiné uniquement pour une utilisation en intérieur de bâtiments avec degré de pollution max. de niveau 2.

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.

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1.6 Carrying instructions

The FlowStar has 2 built-in photo multipliers and a heavy shielding. These are mounted in the front of the instrument. This means the center of gravity (cog) is located more to the front of the instrument.

This must be taken into account when carrying the FlowStar². To allow a comfortable transport of the FlowStar always grab the instrument at the first third of the front to get it balanced properly.

The weight of the FlowStar² is approx.16kg so use caution when carrying it.

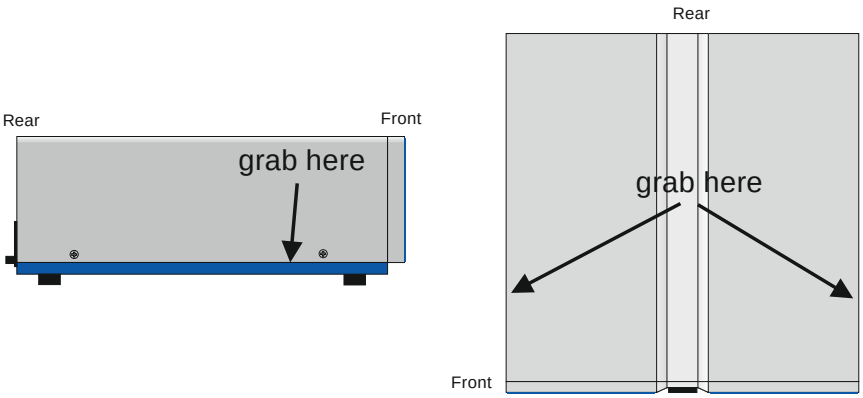


Figure 1: Grabbing Positions

1.7 Overview

The Radioflow Detector ***FlowStar²*** may be connected to any chromatography data system. A special interface ensures that the signals transferred to the data station (HPLC controller/integrator) will be processed just like those from a UV monitor.
The Radioflow Detector is employed for the measurement of all liquid sources commonly used in radiochromatography. HPLC measurements detect α, β and γ radiation using various measuring cells which are installed downstream of separation column and mass detector (UV monitor).
The microprocessor performs the following control and process functions: setting of energy channels, data reduction of radioactivity signals, elimination of noise and luminescence events through coincidence circuit, control of scintillator pump, ratemeter function, DPM calculation, background subtraction.

The USB interface supports external control of the monitor and parameter input via computer.

Figure 1 show a typical configuration and illustrates the ***FlowStar²*** functions.
The measured radioactivity is output as analog signal (0 - 1 Volt) at the ratemeter output after data reduction by a 16 bit digital/analog converter. This output has to be connected to the available HPLC controller/integrator system.

The liquid scintillator pump (LB 5037) is either controlled by the Radioactivity Monitor ***FlowStar²*** (through serial port) or it is controlled manually.

The monitor is operated via the touch screen monitor on the front panel of the device (see Figure 2). All parameters are set and the measurements evaluated and displayed graphically and numerically via the ***FlowStar² software***.

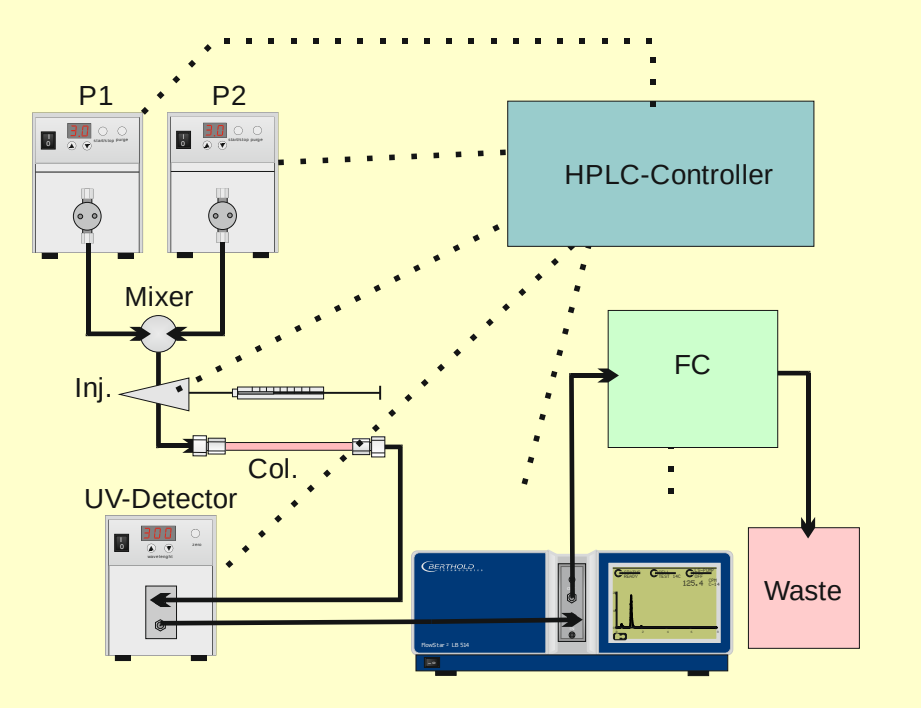


Figure 1: Configuration with solid cell

We have selected the following setup for illustration:
Two eluates are supplied via two pumps (P1 and P2) and mixed. The sample is injected by an injection valve (Inj.). In the column, the sample is separated into individual components (peaks), passed through the UV detector and then to the cell in the HPLC monitor and measured. The eluate flow is split via the 2/3-way valve (V) and the radioactive peaks are collected in the vials of the fraction collector (FC).

- - - - - - = connections for commands and data transfer (control lines)
_____ = capillary supply lines for eluate, sample and scintillator

FlowStar² comprises the following system components:

- a microprocessor for data reduction of the radiation channels, waste or fraction collector control and scintillator pump control
- two detectors working in coincidence
- a measuring cell located between the detectors. A number of different measuring cells, which can easily be exchanged, are available for various applications.

Further Berthold devices may be connected depending on the measuring configuration (see Figure 1):

- scintillator pump LB 5037 required for the admixture method
- T-mixer (also for the admixture method) and, if necessary, a mixing chamber (static mixer)
- Analytic splitter (AS).
- Waste valve to separate radioactive and non radioactive waste.

The following devices may be connected to the HPLC monitor:

- PC data system (**USB**)
- External start control (e.g. injector valve or autosampler etc.)
- Recorder (**Analog Output**)

Connection of PC
FlowStar² can be connected to a PC via the USB interface. In this case the computer controls the operation of the monitor. Additional functions such as data reduction and storage of parameter sets are now available. If the computer is in charge, no data can be entered via the touch screen monitor.

In the following chapters we will describe the individual system components in detail:
Radioactivity Monitor
Measuring cells
Scintillator pump
Analytic splitter
Waste valve

The operation of ***FlowStar²*** and the individual parameters will be described in Part III: *Operation of FlowStar*.

2. HPLC Monitor

*Figure 2: Measuring cell module and Radioactivity Monitor **FlowStar²***

FlowStar² includes the following connection options , as illustrated in Figure 3 (rear view) and Figure 2 (front view with measuring cell module):

- *"**Ratemeter**": Analog outputs of the 2 radioactivity* channels for connection of an HPLC controller/integrator, a data system or a recorder.
- *"**Ext. Control**": External control.* Connection of start signal from injector valve or autosampler.
- *"**Scint. Pump**": Control of scintillator pump LB 5035* with 9-pin Sub-D socket.
- ***Measuring cell** as module on the front side* (Figure 2).
- *2 analog inputs* (Figure 3, **Analog In 1** and **Analog In 2**).
- Connection with a PC or another data system can be established via the *"**USB**"* interface to save and analyze the measured data and to control ***FlowStar²***.

All connections are established on the rear panel of the device. Mains connection (**Mains**) and mains fuse (**Fuse**) are also located on the rear panel**.**

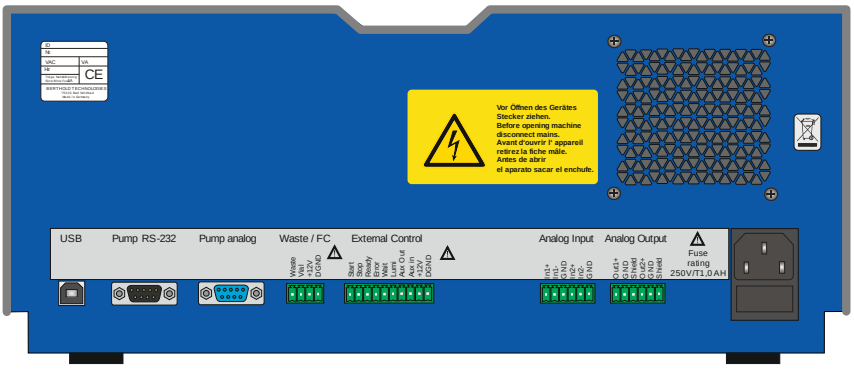
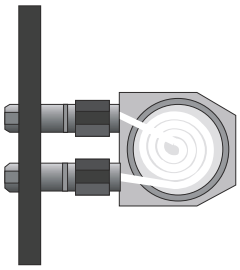


*Figure 3: Rear panel of **FlowStar²** with connection ports and terminal connections*

2.1 Measuring Cell Module



The measuring cells are designed as modules and are therefore easy to replace. They are inserted into the instrument from the front and fixed by two screws.
Each measuring cell includes a chip that offers two functions:

- When it is inserted, it automatically established contact when the measuring cell is fixed with screws, and cuts the contact again as soon as the screws of the measuring cell are released. This ensures that the high voltage is turned on only when the counting chamber is closed.
- It informs the system about the parameters of the measuring cell. This ensures that only methods can be used during a run which are suitable for this measuring cell.

An O-ring seals off light. The measuring cell itself is usually a spiral-shaped thin Teflon hose through which the eluate is flowing. The radioactive labeled substances in the eluate flow are detected by means of scintillator, either solid scintillator, located stationary in the measuring cell, or liquid scintillator which is added to the eluate. The measuring cell is located between the photo cathodes of two photomultipliers operating in coincidence.

Depending on the measuring method either

- solid cells (YG-cells),
- admixture cells (Z) or
- cells for Gamma or Cerenkov radiation (BGO, J, MX, or Z cells)

can be employed in different volumes.

Automatic settings
The nuclide and further parameters are entered in the dialog mode. Then – depending on the measuring cell used – the coincidence time is set according to the decay time of the scintillator used (±100 ns for solid and ±30 ns for liquid scintillator) and the corresponding energy window is activated.

2.2 Radioactivity Detector

Nuclear radiation is detected using a scintillation counter, plus a coincidence circuit.
The eluate passes through the measuring cell located between the cathodes of two photomultipliers. The radiation in the eluate excites luminescent substances (e.g. scintillator grit, liquid scintillator) and triggers flashes of light whose photon number is proportional to the radiation intensity. These emitted photons release photoelectrons on the photosensitive layer of the photomultiplier. These electrons are accelerated by the high voltage applied and move toward the anode; their impact on a dynode system generates further electrons. The resulting current pulses are then amplified and selected in the discriminator. The main task of signal evaluation is to detect counts caused by radioactive decays and to distinguish them from counts caused by

- Luminescence
- Photomultiplier noise

This distinction is achieved by means of the coincidence circuit of two photomultipliers and through compensation of the pulses caused by random coincidence.

If the photomultipliers does not work properly, please contact the Berthold Technologies service (Service hotline: 07081-177-111).

The detector must not be modified by the user!

2.3 Monitor Connections

All connection ports of ***FlowStar²*** are located on its rear panel (Figure 5), except for the measuring cell module (Figure 4) with the ports for the steel capillaries passing the eluate to [3] and from [4] the measuring cell. The measuring cell is inserted on the front panel and fixed by screws. This establishes contact between the chip integrated in the measuring cell and the Monitor. The chip is read when the Monitor is powered up or when the Monitor is running: the high voltage for the photomultiplier is turned on automatically and the measuring cell parameters are transferred to the Monitor.
The high voltage is turned on only if the measuring cell has been fixed correctly. This protects the photomultiplier against incidence of light.
An measuring chamber outlet connector (6) is also located underneath the measuring cell. This should be connected to a waste container. In case of a cell leak the leaking liquid is guided out of the measuring chamber through this tube.

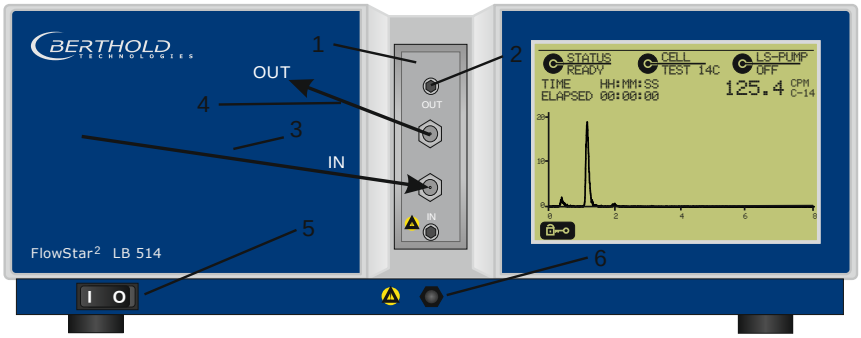


Figure 4: Front view of Radioactivity Monitor

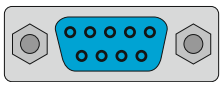
1	=	built-in measuring cell
2	=	fastening screw
3	=	inlet capillary (IN)
4	=	outlet capillary (OUT)
5	=	ON/OFF switch of monitor
6	=	measuring chamber outlet connection

USB

USB connection

A PC can be connected via the **USB** interface. The connection cable is included (USB A to USB B). Before connecting the FlowStar to a PC make sure the driver software was installed properly before. Otherwise the device will not be discovered and installed in the Windows device manager.

Pump analog



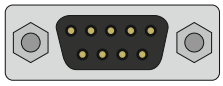
Scintillator pump analog

Pin assignment

Pin 1:		Ground
Pin 3:	out	Control of the output from 0-10 V = 0 - 10 ml/min
Pin 4:	out	Pump on
Pin 5:	out	Pump on/off (low = start, high = stop)
Pin 8:	out	Pump off
Pin 9:	out	+15 V

Please refer to the information in Part III, Chapter 4 for control of the scintillator pump LB 5037 via ***FlowStar²***.

Pump RS-232



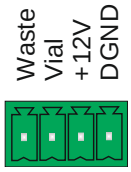
Scintillator pump RS232

Cable no.: 26204 (Zero Modem cable)
(included with the scope of supply of the pump)

The scintillator pump LB 5037 is controlled through the serial port of the FlowStar². The socket (9 pin male sub-D) is located next to the USB connector. The connection cable is included with the scope of supply of the pump.
The 9-pin Sub-D socket labeled **Pump analog** is the connection socket for the control of the older scintillator pump LB 5035-3 or other pumps controlled by an analog pump flow signal.

Waste / FC / FC

Waste / FC



The fraction collector control and the waste valve are connected to the 4-pin socket **Waste/FC**. The wiring is made depending on the available hardware using the cable supplied (loose ends).

Pin assignment

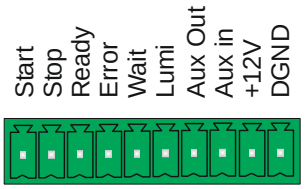
Waste	Waste valve output (open collector)
Vial	Vial advance (open collector)
+12V	+12V supply voltage (max. rating 100mA)
DGnd	Digital ground

External Control

Cable no.: Cable with loose ends for wiring as you choose

For external control of ***FlowStar²*** via a HPLC control system or an autosampler.

External Control



Pin assignment

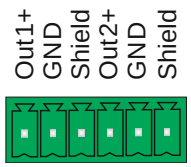
Start:	Start signal. A measurement is started by closing (edge) the **Start** and **DGnd** inputs.
Stop:	Stop signal. A running measurement will be stopped if 0V are applied (open collector).
Ready:	Ready signal. Applied to (0V) if the device is ready for measurement (open collector).
Error:	Error signal. Applied to (0V) if a device error (e.g. leakage) exists (open collector).
Wait[1]:	Wait signal. Indicates that the detector is ready to measure and waits for a start signal. This signal can be used to trigger an autosanmpler to inject a sample.
Lumi:	High Luminescence signal: This signal is active in case a high luminescence state is detected (if enabled in the system settings. A high luminescence usually indicates mixing problems (in case of liquid scintillation method) or a light leak due to improper tubing.
Aux Out	Auxiliary output. Can be controlled through the controller software.
Aux in	Auxiliary input. An external status signal can be monitored at tis port. Status is available though the controller software.
+12V	Supply voltage (max.rating 100mA)
DGnd	Digital ground

[1] Available in a later firmware revision.

Analog Output

Analog signals which are proportional to the radioactivity are output to the analog output pins for the integrator or an external evaluation system. The scale is set on the **Methods** menu (Methods parameters page 2). The information in the dialog refers to 1 Volt: e.g. **Value at 1 V [Unit / V]:** 1000, then 1000 cpm correspond to 1 Volt at the output. The output signal goes linear to about 2.5V (i.e. 300%), so that – in our example – count rates up to 2500 cpm will be transferred. Values above 2500 cpm will be cut off.
An A/D converter may be connected to this output to convert the radio signal into a HPLC system compatible information. The wiring is made depending on the available hardware using the cable supplied (loose ends) or HPLC system specific cables.

Analog Output



Pin assignment

for the 1st counting channel

Out1+	"+" output signal
GND	"-" or Analog ground
Shield	shielding pin

for the 2nd counting channel

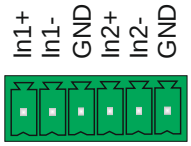
Out2+	"+" output signal
GND	"-" or analog ground
Shield	shielding pin

If only one counting channel is activated, the output signals are always applied to the 1st counting channel.

Analog Input

External detectors, for example, UV or fluorescence detectors, can be connected to these inputs. The input range is at max. +/-5V. The wiring is made depending on the available hardware using the cable supplied (loose ends).

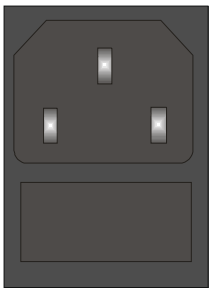
Analog Input



Pin assignment

for the 1st analog channel

In1+	positive input channel 1 (differential signal)
In1-	negative input channel 1(differential signal)
GND	Analog ground
In2+	positive input channel 2 (differential signal)
In2-	negative input channel 2(differential signal)
GND	Analog ground



Mains Supply with Fuse

Cable no.: ID. 80672

The device is connected to mains via the power socket (Figure 5 **Mains**) (DIN 49457 B, CEE 22) and the supplied power cord or another, suitable power cord. The device can operate with a power supply from 100 to 240 VAC (+/-10%), 50/60Hz.

Before establishing the connections please note that the mains supply indicated on the instrument (see label next to power socket) is compatible with the local power supply.

The mains fuses (Figure 5 **Fuse**), located next to the power socket.
Fuse rating:
250V/T1,0 AH (5 x 20 mm)
Protection type: Protection class I according to VDE 0100.

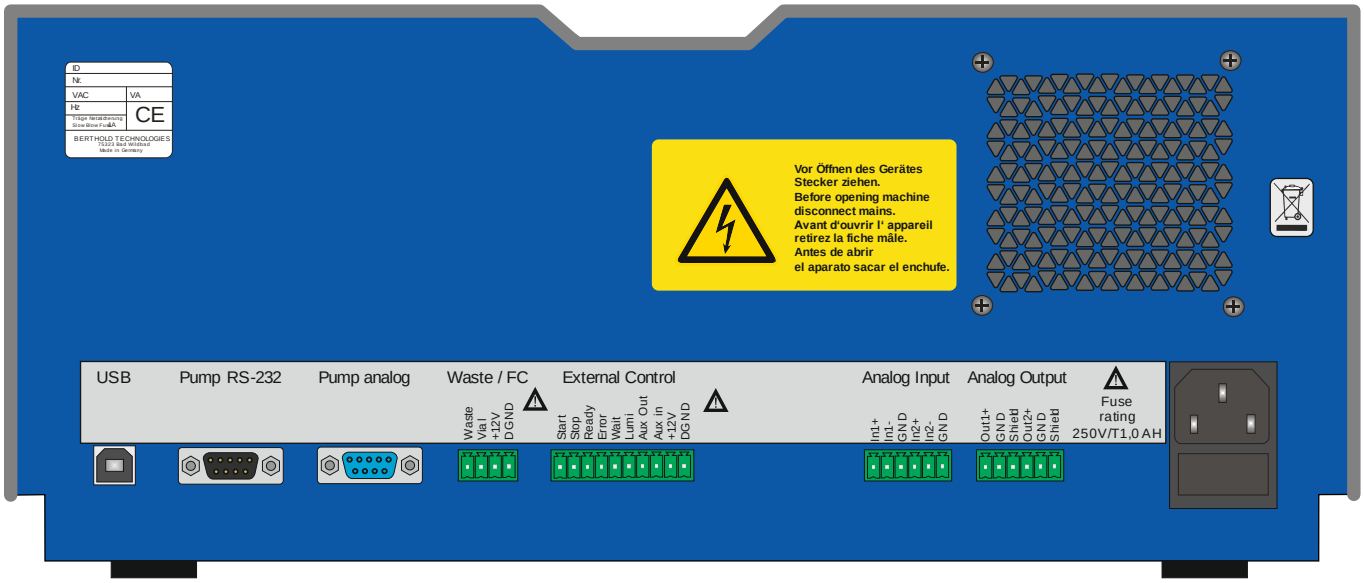


*Figure 5: Rear panel of **FlowStar²** with connection ports and terminal connections*

2.4 Leakage Sensor

FlowStar² includes a leakage sensor at the lowest point in the counting chamber and a hose to drain liquid.
The leakage sensor responds to minor amounts of liquid which may escape from a defective measuring cell. In this case, a warning message is displayed, the scintillator pump stops immediately and an error output signal will be provided.

Separate the device from mains immediately!

Clean the device thoroughly. Depending on how much liquid has escaped, you have to observe special precautions! See also chapter 3.5.
Liquids from admixture cells often contain aggressive substances in addition to the radioactivity which have to be removed completely as they can also reach the PMTs. Otherwise, they will continue to have a harmful effect on the device and may ultimately damage it. For this reason, the device should be cleaned by BERTHOLD TECHNOLOGIES service personnel.
In **solid cells** the eluate includes radioactive substances which have to be removed carefully, taking special precautions.
The leaking liquid is guided to a waste container using the outlet connector located below the measuring cell at the front panel.
To connect the outlet to a waste container use the provided black tubing.

Do not block the emergency outlet at the instrument.

In order to assure a complete system shutdown connect the FlowStar² error output signal (see 2.3) to the HPLC system- or pump shutdown input.

3. Measuring Cells

A number of different Berthold measuring cells are available for radioactivity measurements in column eluates; all common sources can be measured with high efficiency with these cells. Depending on the measuring method the cells are of different design (see Table 2).

3.1 Selection of Measuring Cells

Measuring cells are often distinguished according to the method of application:

- *Heterogeneous method*: eluate and scintillator are separated (solid scintillator). The eluate is completely available after the measurement once it has passed through the cell.
- *Homogeneous admixture method:* the column eluate is mixed with liquid scintillator and the resulting "cocktail" passed through the cell. After having passed through the cell, the eluate is only available as a mixture.

For our purposes, we select a classification that is based on practical considerations: on different applications and the resulting structure of the measuring cells:

(a) Cells with solid scintillator (YG, MX)

(b) Homogeneous admixture method (Z)

(c) Special methods for Gamma radiation (J and BGO)

(d) Measurement of high-energy beta radiation using the Cerenkov effect without scintillator (Z)

Identification of the cells

The cells are marked on their front plate by letters and numbers, which have the following meaning and order: Example: YG-150 U 5 D

YG
150
S
5
D

The first letter group identifies the cell type or the scintillator:
YG = Yttrium glass, Z = admixture cell, J = iodine,
BGO = external BGO scintillator, MX = MX scintillator.

The numbers indicate the cell volume in µl.

A possibly following letter indicates a surface treatment:
U = untreated, S = surface treatment

The following digit indicates the cell generation:
5 means 2"-photomultiplier counting chamber with cell ID chip starting with device LB 513 in contrast to older series. The FlowStar^2 can only be used with measuring cells of generation 5 and higher.

The last letter group identifies special applications:
P = preparative cells, D = cells with wire inside, M = cells for Micro-HPLC with low dead-volume in- and outlets

Example: **Z-200 6M** means: **admix cell**, **volume 200 µl**, **type 6, mircobore**.

Cell	ID No.	Remarks	Max. pressure (bar)
YG-50 S 5 P	50152	Preparative cell with surface-treated scintillator for high activities. Max. flow rate: 20 ml/min	50
YG-150 U5D YG-400 U5D	50157 50137	Cells with Yttrium/glass scintillator. Very robust analytical cells with highest sensitivity. Max. flow rate: 3-4 ml/min	50
YG-150 S5D YG-400 S5D	50158 50138	Cells with surface-treated Yttrium/ glass scintillator. Very robust analytical cells with highest sensitivity. Max. flow rate: 3-4 ml/min	50
YG-10 U6M YG-40 U6M YG-75 U6M YG-150 U6M	55216 53263 53261 55211	Cells with Yttrium/glass scintillator and small volume and low dead-volume in- and outlets for Micro-HPLC application. Max. flow rate: 1-1.5 ml/min.	100
YG-10 S6M YG-40 S6M YG-75 S6M YG-150 S6M	55215 53262 53259 55210	Measuring cell with surface-treated Yttrium/ glass scintillator and small volume and low dead-volume in- and outlets for Micro-HPLC application. Max. flow rate: 1-1.5 ml/min. Cells with Yttrium/glass scintillator.	100
Z-500-5 Z-1000-5	50154 50153	Analytical admixture cells with highest sensitivity. The cells can simultaneously be used for ^{32}P measurement via Cerenkov effect.	15
Z-20 6M Z-50 6M Z-100 6M Z-200 6M Z-500 6M	64345 58178 54672 54419 55196	Admixure cells with small volume and dead volume in- and outlets for Micro-HPLC application.	100
MX-20 6 MX-50 6 MX-100 6 MX-200 6 MX-500 6	58534 54421 61886 54303 61887	Special cells for measurement of PET isotopes.	100
J-1000-5	80080	For low-energy Gamma sources with external scintillator	15
BGO-X	51114	Cells with external BGO scintillator for measurement of Gamma sources with replaceable Teflon loops: 5 µl, 30 µl and 150 µl.	15

Table 1: Data of measuring cells

a) Measuring cells with solid scintillator

They consist of thin Halar or Teflon hoses filled with fine-grained scintillator grit (glass, Yttrium glass as scintillator). The scintillator is located stationary in the cell through which the eluate passes. Both ends of the cell incorporate frits to prevent that the scintillator grit is flushed out of the cell. A solid cell generates a certain backpressure which is dependent on the flow rate and the viscosity of the eluate. In analytical cells the backpressure is approximately 4 - 6 bar/ml methanol (for further information see Part IV of this User's Manual)

Application: For Alpha and Beta labels: ^{3}H, ^{14}C, ^{35}S, ^{90}Sr etc.

Volume: 10 - 400 µl (larger volumes on request)

Filling material: Glass, Yttrium glass
The grain size is optimized with regard to efficiency and backpressure: Analytical cells have a small, preparative cells a large grain size.

Handling: Easy handling, good tolerance of all eluates, rare memory effects, removal of possible contamination by decontamination solvents possible without any problem.

Identification: Measuring cell label - Example:
YG 150 U5D (Yttrium glass solid cells with 150µl volume)
YG 40 S6M (surface treated Yttrium glass microbore solid cells with 40µl volume)

Caution:

The outlet capillary for solid cells should have an interior diameter of at least 0.5 mm.

Please read Chapter 4.2 Connecting Steel Capillaries before connecting a solid cell.

b) Admixture method
Liquid scintillator is continuously added to the column eluate, the resulting mixture passed through a cell and measured. Advantages of this method: virtually no memory effects and a high counting efficiency for all nuclides (e.g. for 3H-labels up to 50%), therefore highest detection sensitivity (E^2/BG).

The following instruments are required for this method:

- Scintillator pump LB 5037
- T-mixer or mixing cartridge with housing
- Possibly, an analytical splitter to minimize liquid scintillator consumption and/or to re-use the eluate
- Liquid scintillator, with certain properties: it should absorb the existing eluate without any problems; the resulting cocktail should remain transparent, without jellification, crystallization or flocculation. Moreover, no mixture gaps or luminescence must occur.

Volume: 20 µl - 1 ml

Handling: No contamination or memory effects, additional devices required.

Identification: Labeling example:
Z 200-6 M (microbore admixture cells with 200 µl volume)

Caution:
The outlet capillary for solid cells should have an interior diameter of at least 1.0 mm.

Please read Chapter 4.2 Connecting Steel Capillaries before connecting an admixture cell.

c) Measurement of Gamma Radiation

Two types of cells are available for the measurement of gamma radiation:

The **J-1000 cell** is used for low-energy Gamma radiation up to 50 KeV and is intended for the measurement of ^{125}Iodine.

Cell structure A Teflon hose spiral between 2 BGO scintillator discs

Volume Cell volumes between 20 µl and 1000 µl are available.

Identification Labeling: J 1000-5 cell type: J = iodine, volume:1000 µl

The **BGO-X cell** can be used for any, even high-energy gamma radiation.

Cell structure Bismuth germanate single crystal, size ∅ 40 x 15 mm with bore-hole. The bore-hole contains a replaceable glass vial cartridge. The cell volume can be selected by replacing the glass vial.

Volume 5, 30 and 150 µl. All 3 measuring loops are included with the shipment.

Identification BGO-X

d) Measurement of high-energy beta radiation such as ^{32}P

^{32}P is easy to detect by means of the Cerenkov effect. Common admixture cells with the desired volume are employed for the measurement of Cerenkov effects. Light pulses are generated in the cells which can be detected with high efficiency. Essential advantage of this method: very low background due to the fact that no scintillator is in the counting chamber. Therefore, excellent detection limit (=E^2/BG).

Identification Labeling example: Z 500-5 or Z 200-6M
Admixture cell with 500 µl or 200 µl volume

e) Tailor-made measuring cells

Measuring cells are also manufactured according to customer's specifications. The options and the information required for production are listed in Chapter 4. Measuring cells can be adapted to a customer's specific needs. For example, cells for preparative applications can be supplied, operating with a flow rate far above 10 ml/min, and a minimum backpressure (of about 2 bar at 10ml/min). The cells are also employed in traditional liquid chromatography (LC). The preparative cells offer only negligible resistance against the atmospherically created flow in a gel-column system.

3.2 Assembly and Disassembly of Measuring Cells

The measuring cells have been designed as a module for easy installation (and replacement) from the front of the monitor (see Figure 6). The module is fixed by 2 socket head cap screws size M 4 x 12 mm. (The screws and the respective screwdriver are included with the shipment.)
Each measuring cell includes a chip that offers two functions:

- When it is inserted, it automatically established contact when the measuring cell is fixed with screws, and cuts the contact again as soon as the screws of the measuring cell are released. This ensures that the high voltage is turned on only if the counting chamber is closed.
- It informs the system about the parameters of the measuring cell. This ensures that only methods can be used during a run which are suitable for this measuring cell.

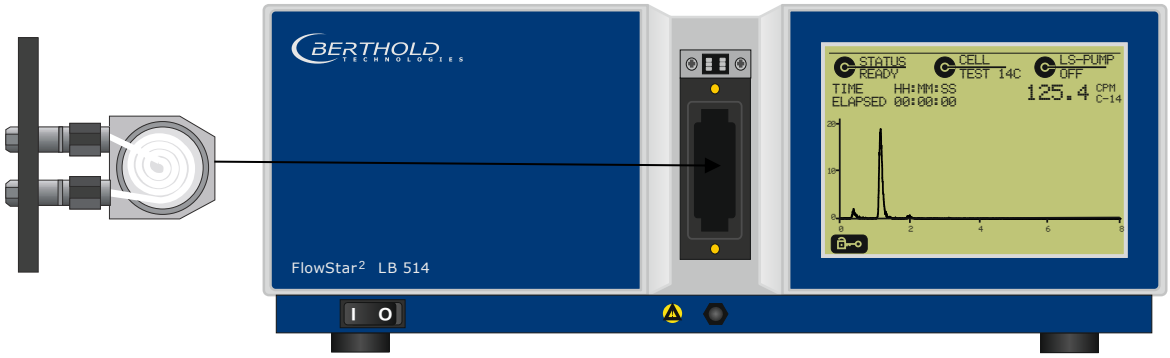


Figure 6: Front view of ***FlowStar²*** *Monitor*

Light seal and incidence of light
An O-ring seal (59 x 2 mm) protects the measuring chamber against light. Make sure the seal is clean and seated properly in the groove of the counting chamber!
The base plate of the cells should be clean and without any alien materials (e.g. labels)!

Spare O-rings are included as accessories.

Excessive incidence of light into the measuring chamber has to be avoided, as otherwise the noise rate of the photomultiplier will increase and drop to the normal level only after several hours. If direct sun light and direct light from fluorescent lamps is avoided, and the chamber is not open for an unnecessarily long time, the instrument will be ready for measurement after just a few minutes.
GT-solid scintillator cells and the Gamma measuring cell, e.g. **J-1000** and **BGO-X**, should also be stored in a dark room and not be exposed to light during assembly in order to reduce phosphorescence. The decay time of photomultipliers and GT cells is dependent on the amount of light it has been exposed to: the more light the photomultipliers or GT cells have "seen", the longer the decay period.

3.3 Connecting Steel Capillaries

Only stainless steel capillaries should be connected to the measuring cell for supply (IN) and disposal (OUT) of the eluate or the eluate cocktail to prevent incidence of light.
Teflon or other plastic capillaries, e.g. PEEK capillaries, do not ensure any light-sealed operation!
When using Teflon capillaries, a 10 - 15 cm long steel capillary piece should be used before each cell in- and outlet; shape it in the form of a spiral or a wave to suppress any light guide effect.

The outlet capillary must always have a larger inner diameter than the inlet capillary! (see Figure 7)

Outlet capillary in solid cell: min. ID ∅ 0.5 mm
Outlet capillary in admixture cell: min. ID ∅ 1.0 mm

The following capillaries are supplied with each instrument:
1 m steel capillary OD 1/16" ID ∅0.5 mm (for supply and disposal)
1 m steel capillary OD 1/16" ID ∅1.0 mm (for disposal)

Please check the capillaries every time you connect new capillaries!

Before connecting a capillary, please check and see that the bore-hole of the steel capillaries is open and the connection sleeves of the measuring cell are clean.
When cutting the length of the steel capillaries, never pinch them off, but always use a capillary cutter, or break the capillary after slightly scratching it by means of a capillary cutter or a feather-edge file!
If the outlet capillary has to be extended, please make sure that no low dead-volume capillary unions will be used. These unions have only a very small bore-hole which leads to a rise in backpressure which may destroy the cell.

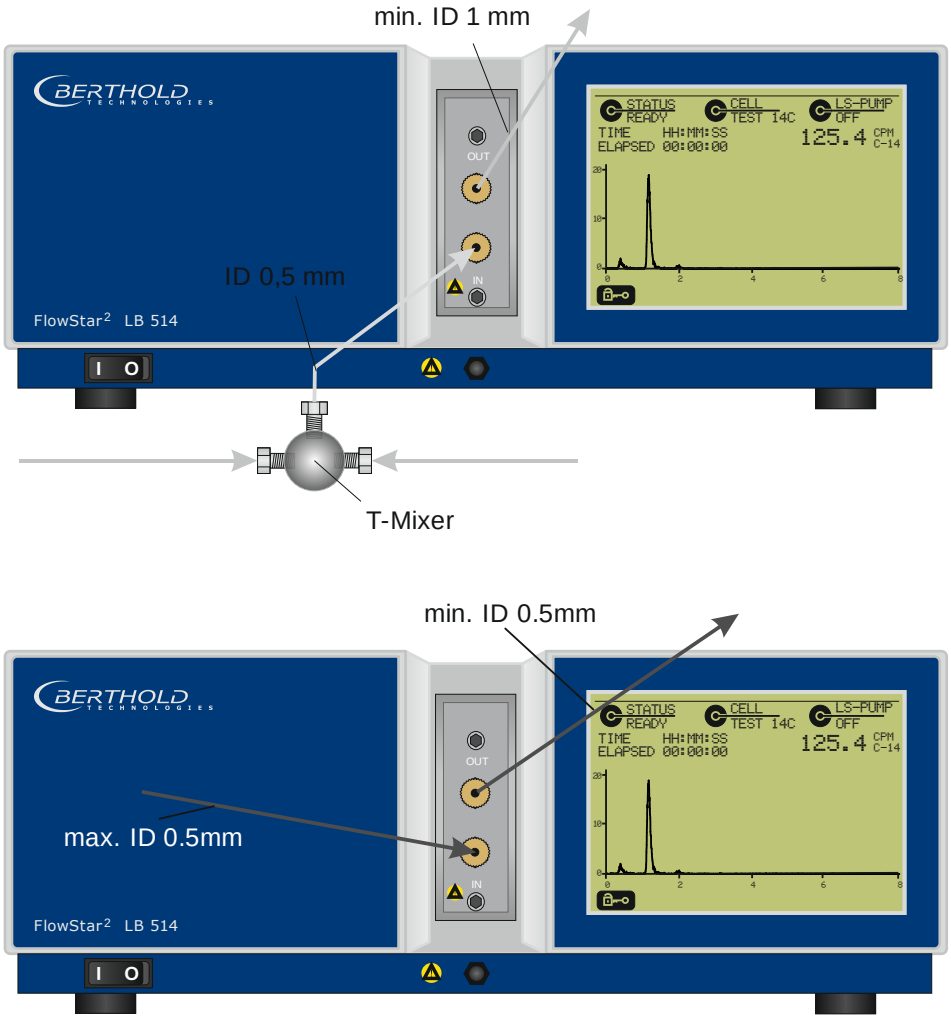


Figure 7: In and outlet capillaries for solid and admixture cells

Installation procedure for fingertight screw fitting

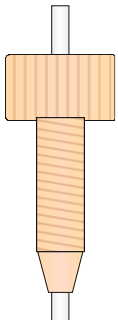


Figure 8: Capillary with PEEK "fingertight" screw fitting

- ❑ Push capillary through the fingertight screw until a piece of it protrudes at the other end.
- ❑ Set screw onto the connecting piece and tighten it slightly.
- ❑ Push capillary all the way into the screw and the connecting piece.
- ❑ Fingertighten screw, make sure it is tight and if you detect any leakage, tighten screw a bit more.

3.4 Cleaning the Measuring Cells

Clean the measuring cells regularly, i.e. between 2 cycles, and flush the system after the last cycle using a solvent. In case of heavy contamination the cell has to be decontaminated; if in doubt, replace it.
The measuring cells are cleaned by means of a PE disposable syringe (2 or 5 ml) as follows:

- ❑ Unscrew the inlet capillary.
- ❑ Prepare a coupling so that disposable syringe and measuring cells inlet can be fitted together accurately. Use a 200 µl pipetting tip (yellow), and cut it on both ends by 8 mm (see page 5-5).
- ❑ Fill the syringe, e.g. with H_2O_2(or with a detergent solution), place it onto the syringe, put the syringe (with coupling) against the measuring cell inlet, and protect the direct vicinity against possible spillage with paper.
- ❑ Inject the liquid slowly.
- ❑ Finally, flush the measuring cell with water, connect the inlet capillary again, and pump the entire liquid out of the system into a waste container.

3.5 Cleaning the Measuring Chamber

If the measuring cell has become clogged, for example by gel formation or bending of the outlet line, the cell may get damaged by the pressure of the HPLC pump, which may be up to 400 bar. As a consequence, eluate will flow into the measuring chamber.
To prevent damage to the photomultiplier, a **leakage sensor** is installed at the lowest point of the counting chamber, which detects and signals even minor amounts of liquid, so that you can take action in due time and prevent possible damage or, at least, kept it to a minimum. Moreover, at this point, an outlet is installed as an **emergency outlet**, to which a hose is to be connected which is led out on the front of the device, so that spilled liquid can be drained to a waste container.

If you notice that liquid has spilled into the counting chamber, separate the device from mains immediately! Clean the chamber thoroughly. Special precautions have to be observed depending on how much liquid has escaped! Wear protection glasses and gloves.

Liquids from admixture cells often contain aggressive substances which have to be removed completely; otherwise, they will continue to have a harmful effect on the device and may ultimately damage it. For this reason, the device should be cleaned by **BERTHOLD TECHNOLOGIES** service personnel as this requires disassembling of the detectors.

In **Solid cells** the eluate includes radioactive substances which have to be removed carefully, taking special precautions. Dry the chamber using a soft cloth or filter tissue. Possible deposits from a radioactive substance are irrelevant for the measurement, since the radiation cannot reach the scintillator due to the limited range of the isotopes involved. Clean the window of the photomultiplier with a soft cloth.
It is recommended to leave the cleaned measuring chamber open over night for drying, covered with a black cloth to avoid incoming light.

Always make sure that the photomultipliers are not exposed to light! Clean them only in subdued light (light bulb).

Check the emergergency outlet after a leakage to make sure no liquid is dried inside and may block the outlet. In case of a blocked emergency outlet tubing please replace the tubing from outlet to waste container.

4. Scintillator Pump

The HPLC pump LB 5037 supplies liquid scintillator for the admixture method. The pump is a high pressure double-head piston pump for constant liquid supply.

More information how to operate the liquid scintillator pump can be found in the pump manual.

4.1 Connections

The connection sockets are located on the rear panel of the pump:

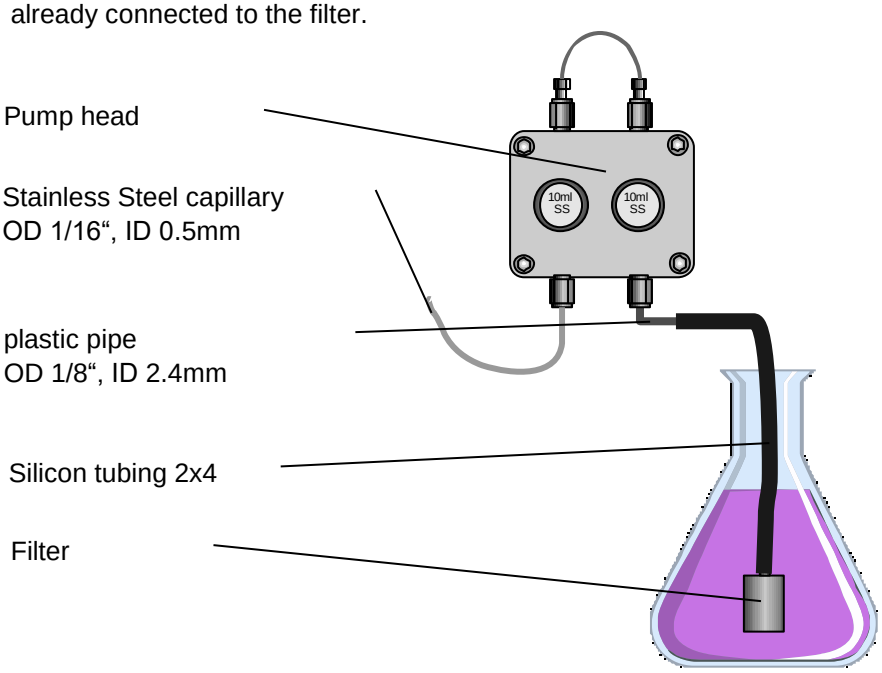
- Power supply (24V)
- The 9-pin Sub-D socket with cable no. 26204 is used for external control via ***FlowStar²***.

The pump head is located on the front of the pump housing. The inlet valve is installed on the bottom right of the pump head, and the outlet valve for the liquid scintillator on the bottom left. How to connect the supply system will be described below. For more information regarding the tubing connection please refer to the pump manual.

Figure 9: Front view of the HPLC pump

4.2 Connecting the Supply System

Supply of liquid scintillator:
Outer diameter: 1/8", ID 2.4 mm
The supply valve is located on the bottom right of the pump head. To ensure a proper function, the supply line should have an internal diameter (ID) of at least 2 mm and not be longer than 1 m. Thin supply lines may cause gas bubbles, for the short aspiration time and the too thin supply line may cause under pressure.
Therefore, the supplied angled stainless steel knee pipe (OD 1/8", ID 2.4 mm) with installed screw fitting and Teflon seal should be used in any case; it should be screwed into the bottom opening of the pump head. Place the 1 m long, black Vinton hose over this knee pipe piece (type Iso-Versinic, OD 4 mm, ID 2 mm, also included with the shipment). The other end is already connected to the filter.



The filter should always be clean. In case of possible gel-like deposits or impurities, clean it using methanol or ethanol. For cleaning, the filter may be dismantled and the filter disc removed and, if necessary, replaced. The pump inlet fitting of the tubing is mounted as shown in the picture below.

Outlet of liquid scintillator: OD 1/16", ID 0.5 mm, length 0.5 - 1m
The outlet valve is located on the bottom left of the pump head. Fix the supplied stainless steel capillary (OD 1/16", ID 0.5 mm, length 1 m) with already mounted screw fitting here. The mixer is installed on the other end.

The length of the stainless steel capillary must not be altered.

The capillary path acts as resistance, so that a slight backpressure (approx. 2 bar at 5 ml/min flow rate) will occur which is required for the exact switch function of the ball valves.

Caution:

- Do not shorten the 0.5 - 1 m long stainless steel capillary.
- Do not use any capillary with less than 0.5 mm internal diameter; otherwise there is the danger that capillaries will clog up.
- When working with a pump, the liquid scintillator container should be positioned below the pump to ensure that a negative pressure is created in the supply line and the inlet valve is closed. However, if the liquid scintillator level is above the pump head, the valves of the pump head are opened so that:
    - a backflow from the HPLC system can take place or
    - liquid scintillator flows even if the pump is not in operation..

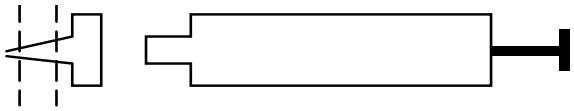
Aspiration of liquid scintillator

Liquid scintillator (or a detergent solvent) is automatically and continuously taken up when the supply line is filled with liquid up to the pump head. If the supply line is not completely filled (e.g. if air bubbles exist) when taking the pump into operation and occasionally after cleaning the pump, a few preparations have to be carried out before starting to aspiration new scintillator.

Two alternatives are possible:

a) New aspiration by disposable syringe

❑ For fast aspiration of the scintillator liquid we recommend using a PE disposable syringe (2 or 5 ml), which is set against the outlet valve of the pump. Since the shape of these syringes does not fit onto the cone of this valve, use a 200 µl pipette tip (yellow) as coupling and cut it on both ends by about 8 mm. Thus you will get an exactly fitting coupling for syringe and outlet valve.



❑ Position the liquid container on a higher level than the pump to utilize the law of communicating pipes. The complete supply line is attached to the pump connecting the liquid container with the pump.

❑ Start the pump by pushing the <**Start/Stop**> button, set the disposable syringe with prepared pipette tips onto the outlet valve, and take up liquid slowly and evenly with the syringe. After a short time the liquid reaches the syringe and you will notice the pressure of the pump pushing the piston of the syringe up.

- ❑ As soon as the liquid gets into the syringe without any bubbles, switch off the pump and place the liquid container on a lower level than the pump head while the syringe is still set. The underpressure created in the supply line automatically closes the inlet valve, so that the supply line (even with turned off pump) remains filled.
- ❑ Pull the syringe – with in-take motion – from the valve to drain off possible residues or spilled liquid.
- ❑ Screw the supplied 60 cm long stainless steel capillary (ID 0.5 mm) with pre-installed connection onto the outlet valve. The pump is ready for operation.

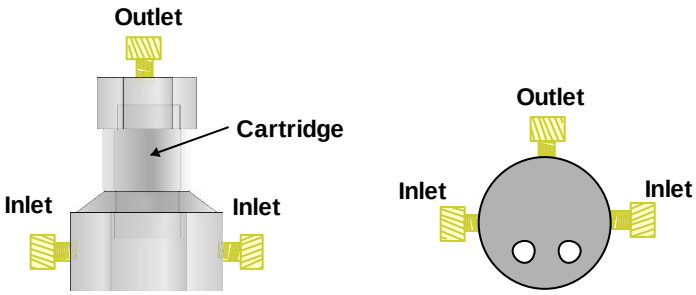
b) New aspiration by scintillator pump

This procedure takes up more time and requires wet valves.

- ❑ The arrangement is the same as described under a):
- ❑ The liquid container is set on a higher level than the pump, the supply line is connected, the outlet not yet.
- ❑ Switch on and start the pump.
- ❑ As soon as the liquid has reached the outlet valve and flows without bubbles, turn off the pump and place the liquid container on a lower level, so that the inlet valve is closed by the underpressure created.
- ❑ Screw the outlet line onto the outlet valve, as described above.

4.3 Mixers

There are 2 different types of mixers available. The t-mixer and a static mixer using 2 different cartridges (50 and 150µl).
Both mixers are used to mix the scintillator flow coming from the pump with the eluate flow.
Due to better mixing capabilities the use of a static mixer is recommended when working with non toxic liquid scintillators.
The pump outlet of the liquid scintillation pump connects to one of the inlets of the mixer. The eluate inlet (usually the tubing coming from the HPLC UV detector) connects to the outher inlet.
The mixer oulet connects to the inlet of the FlowStar² measuring cell. The T-mixer has a special capillary at the outlet, the static mixer uses standard tubing (OD 1/16", ID 0.5mm)



Static Mixer **T- Mixer**

The static mixer consists of 3 parts:

- ❑ Lower housing (inlets are located here)
- ❑ Upper housing (outlet)
- ❑ Cartridge (50 or 150µl)

When using the static mixer, assemble the unit by inserting the cartridge into the lower housing part and close it tight with the upper housing. The in- and outlet connections can be found on the image above.
To avoid mixing problems remove and clean the cartridge after a while in an ultrasolic bath.

5. Analytic Splitter

5.1 Description

The use of the ***Analytic Splitter*** (Figure 10) requires a configuration (admixture method) as illustrated in Figure 11.

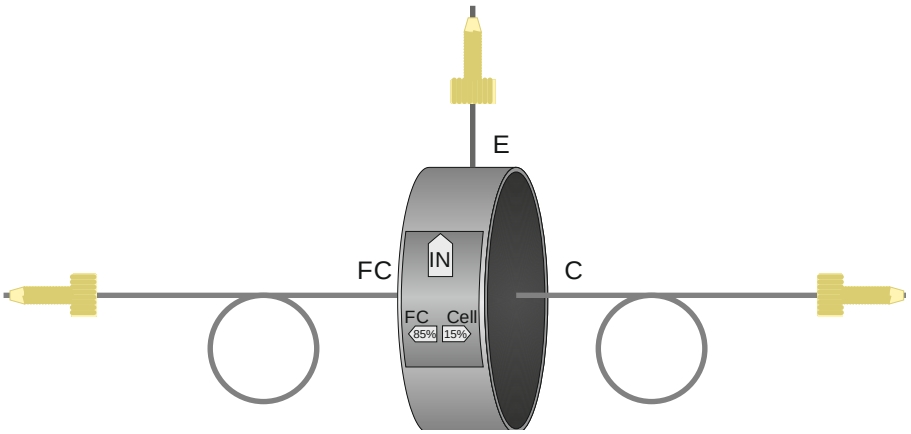


Figure 10: Analytic splitter AS-15
E = Eluate input; FC = capillary to fraction collector; C = capillary to measuring cell

The ***AS-xx*** splits off a certain fixed percentage (xx) for the radioactivity measurement. This partial eluate flow is mixed with liquid scintillator by the T-mixer (T) and passed on to the admixture cell of the HPLC Monitor. The other share of the eluate is passed on to the fraction collector (FC).
Standard versions of the ***AS-xx*** have a splitting ratio of 15%, 25%, 33% and 50%. These percentages define the share of the eluate which is passed on to the radioactivity cell.

Due to the very thin capillaries, the system generates a backpressure of approx. 5 bar at the input (E) of the Analytic Splitter at a flow rate of 1 ml/min (water/viscosity = 1).
The ***AS-xx*** operates without any problem in the flow range from 0.5 to 4 ml/min, and a possible change of the viscosity (gradient) does not affect the splitting ratio.

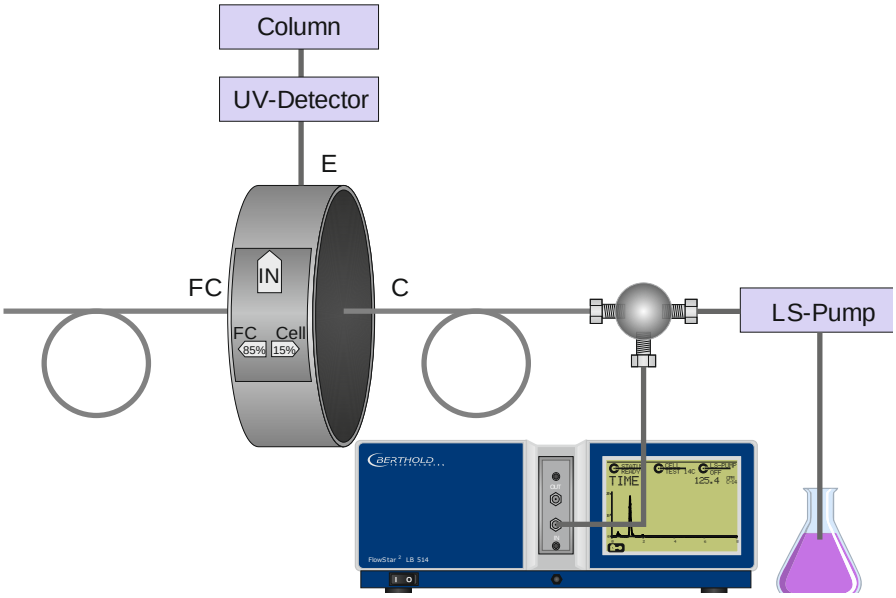


Figure 11: Configuration with Analytic Splitter

The ***AS-xx*** splits off a certain fixed percentage (xx) of the eluate flow toward the measuring cell (C). It is passed on to the T-mixer (T) and mixed with liquid scintillator (LS). This cocktail is then passed on to the admixture cell (Z-xxx) of the HPLC Monitor for radioactivity measurement. The other share of the eluate (100% minus xx) is passed on to the fraction collector (FC).

Diameter and length of the capillaries

The outlet capillaries FC (fraction collector) ***and C*** (cell; or connection to T-mixer [T]) ***must not be reduced in length and increased only by a Teflon capillary!***

When using a stainless steel capillary, it must have a larger internal diameter.

Caution: Total flow rate ≤2 ml/min

With a total flow rate from cocktail (liquid scintillator + split off elate) of < 2 ml/min, use a 60 mm long stainless steel capillary with an internal diameter of 0.5 mm between T-piece and measuring cell.

When using longer or wider capillaries, this may lead to tailing (peak deformation) or double peak formation!

With low cocktail flow rates (< 2 ml/min) the distance between T-mixer (T) and measuring cell should be fairly short.

When setting up the configuration, please check if the connected detector (UV, IR, fluorescence, etc.) is capable of withstanding the backpressure generated. If this is not the case, connect the detector after the ***AS-xx***, i.e. between ***AS-xx*** (FC output) and fraction collector (FC) (see Figure 12).

Higher flow rates (preparative range 4 - 20 ml/min) require a preparative splitter; please specify your parameters: solvent, viscosity, flow rate, required splitting ratio.
For special applications or individual requests please contact our Bad Wildbad headquarters or your local Berthold representative.

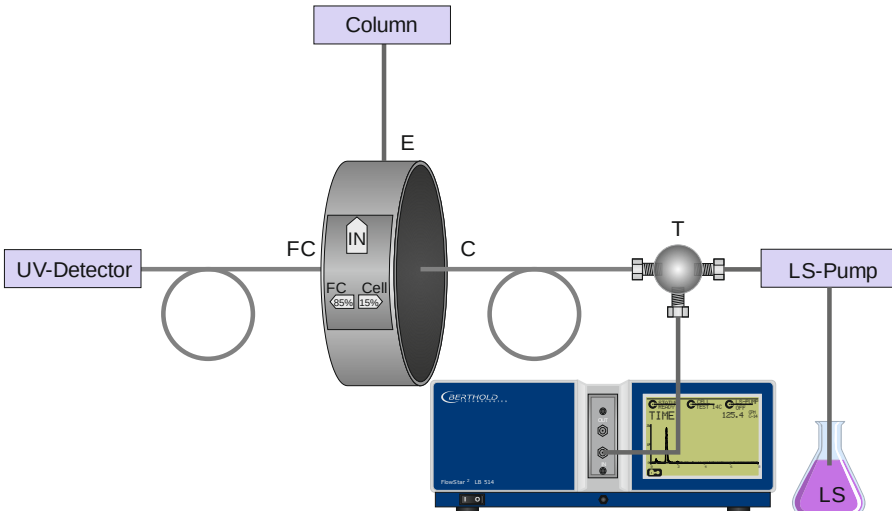


Figure 12: Configuration with subsequently connected UV detector

If the backpressure created by the ***AS-xx*** should cause a problem for the UV detector, you may connect the detector between ***Analytic Splitter*** and fraction collector (**FC**).
The capillaries **C** and **FC** have an internal diameter of **0.1 mm**. The capillaries after the T-mixer to the admixture cell should have an internal diameter of **1 mm** at a total flow rate of **> 2 ml/min**, and **0.5 mm** at a total flow rate of **< 2 ml/min**. In addition, the length of the supply line should be cut to **60 mm** when the flow rate is **< 1.5 ml/min**.

5.2 Installation

Do not increase or reduce the length of the capillaries which are firmly connected to the ***AS-xx*** at the outputs FC and C. Install the connections carefully, since the internal diameter is only 0.1 mm.

- **E** (eluate supply) **0.25 mm ∅ ID**
  Use the ready mounted plastic sealing cone with screw fitting. Replacement in case of a possible defect or leakage in the sealing cone is quite simple. Please note that no chips or other particles enter the ***AS-xx***, for this may block the capillaries.
  The silicon hose rings located before and after the screw fitting serve only for fixing the screw fitting.
- **FC** (fraction collector) **0.1 mm ∅ ID**
  A 20 mm long silicon hose piece is attached to the end of this capillary. Insert a Teflon hose (1/16") of appropriate length into the silicon hose to pass the eluate on to the fraction collector.
- **C** (measuring cell) **AS-xx → T: 0.1 mm ∅ ID; T → measuring cell: 1 mm ∅ ID**
  Use the capillary spiral attached to the ***AS-xx*** as a connection to the T-mixer (T). Here the split off eluate share is mixed with liquid scintillator, transported to the radioactivity measuring cell where it is measured. Due to the viscosity of the cocktail the in- and outlet capillaries (after the T-Mixer) should have an internal diameter of 1 mm and be fairly short (see also page 34) – with a total flow rate >2 ml/min. For this purpose Berthold Technologies supplies a 1 m, 1/16" capillary with an internal diameter of 1 mm together with the HPLC Monitor.
  For a total flow rate of **≤2 ml/min** the supply line from the T-piece to the measuring cell should have a max. **60 mm** long stainless steel capillary with an internal diameter of **0.5 mm**.

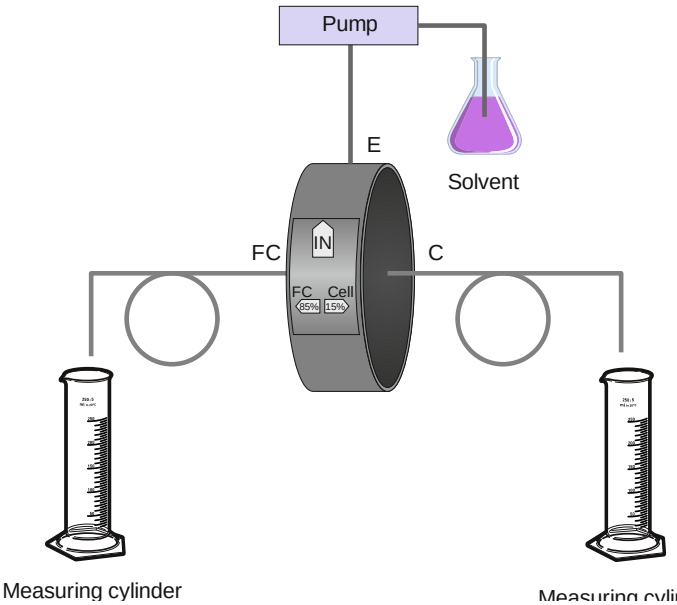


Figure 13: Checking the splitting ratio

A solvent is pumped into the eluate input of the ***Analytic Splitter*** by a pump and the splitting ratio checked using 2 measuring cylinders.
Caution! The flow rate must be absolutely free of pulsation. We recommend connecting the Analytic Splitter directly after the column.

5.3 Inspection and Trouble Shooting

If the ***AS-xx*** does not work with the splitting ratio indicated, check the ***AS-xx*** using the illustration above (Figure 13). Let the pump run for some time, then read off both measuring cylinders. If the liquid is not distributed in both cylinders in the splitting ratio indicated, this may be due to contamination of the capillaries or clogging of the splitter.

Caution: Carry out this check only with an absolutely pulsation-free pump, since any possible pulsation or ripples on the liquid flow may lead to false results of the measurement!

Remedy: Using a complete HPLC system (pump, pulsation reducer, column), check if the pulsation on the high pressure side has already been reduced.

Blocking or clogging in a capillary (FC or C)

With eluates having a high buffer concentration it may happen that the thin capillaries (ID ∅ 0.1 mm) may be blocked by crystallization.
Remedy: Flush the blocked capillary (FC or C) from the rear end with water and collect the water at outlet E (Figure 14).

Blocking the separator

It may happen that the column clogging is leaky and that some particles in the separator may cause a blockage. In most cases, the particles are 3 - 5 µ large and pass through the ***AS-xx*** without any problems. If irregularities occur, proceed as follows:
Alternating flushing of the capillaries FC and C of the ***AS-xx*** with solvent from the rear end (see Figure 14).

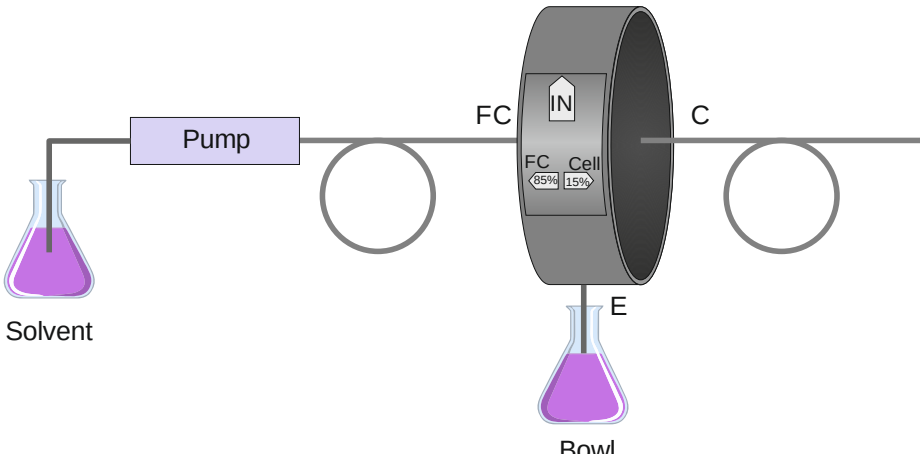


Figure 14: Cleaning capillary or separator

6. Validation using 3H- und 14C-Test sources

The ^{3}H and ^{14}C-Test sources are specially developed to check and validate the function of the FlowStar². The ID chip inside the test cell stores all relevant information which will be transmitted to the FlowStar² when needed. These test cells contain a sealed radioactive source. The source is sealed in a plastic container and leakage tested. The radioactivity of the test cell is far below the permitted limit. It might be required to register this cell if you have a handling permit for radioactive sources. The nominal activity of the ^{14}C test cell is around 200Bq. The nominal activity of the ^{3}H test cell is about 6kBq. Precise activities can be found in the corresponding cell certificate.

Structure
A thick-walled plastic cuvette in a metal cell inset is filled with scintillator and sealed on both sides. The radioactivity (^{3}H or ^{14}C) was adsorbed on the surface of the solid scintillator in a stable form. The cuvette was seal-tested in accordance with 150/DIN 9978 (draft) (DIN 25426, see certificate).

Storage
The test source should be stored well protected in a dark room.

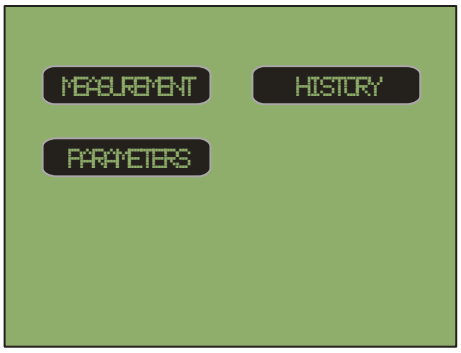
Procedure
An initial validation is necessary when the test source is used for the first time. This initial validation measures the variation between the nominal value of the test source and the measured value of the detector and specifies an initial nominal value. This variation is due to instrument / detector variations mainly caused by the used photo multiplier tubes (PMT). If another test source was used before the validation history must be reset since a validation is always bound to a specific test source serial number. This can only be done through the Berthold service.

In case of further validation measurements the results are compared with the initial nominal value. If the variation is within the defined tolerance level the validation is passed This procedure ensures a constant operation and detects possible instrument problems. The Validation can be performed with the ^{3}H or ^{14}C test sources (own, home made gamma source optional) as well as the background cell. Since the background is depending on the local conditions (cosmic radiation etc.) variations in the background could occour.

6.1 Checking the FlowStar² with test sources

- ❑ Remove cell.
- ❑ Install test source (^{3}H or ^{14}C) in place of the measuring cell. Please make sure that the cell and the test source will not see any light! This will cut down the decay period.
- ❑ Start the validation measurement (Validation | Measurement | <Start>) and wait until the measurement is finished.In case of a passed validation the measured values can be saved in the history. If the validation failed check the instrument and repeat the measurement.

Procedure and parameter entry
Select **Validation** from the main menu. This entry is only visible if you are logged in as supervisor. The following menu appears:

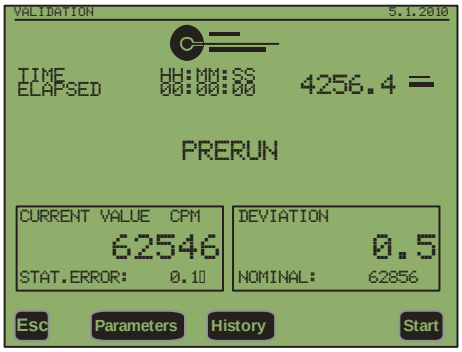


- **Measurement**
  Validation measurements are handled through this menu.

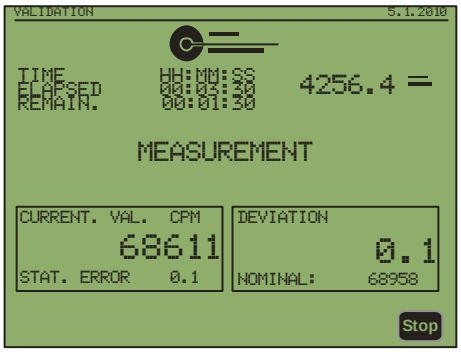
- **History**
  Earlier, successful validation measurements can be revied in the Validation history.

- **Parameters**
  Validation parameters such as tolerance levels and measuring time are defined in this menu.

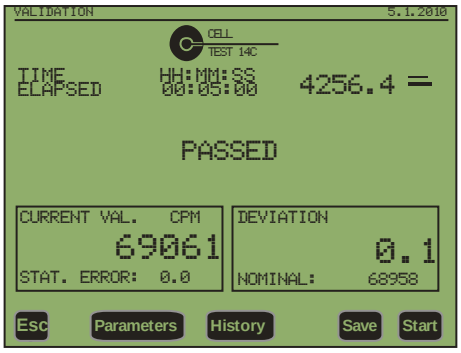
Measurement
When selecting **Measurement** from the validation menu the window to perform validation measurements pops up.



The instrument is in prerun mode. By pressing **<Start>** the validation measurement starts and the current (upper right corner) as well as the integrated value (lower left corner) and the deviation from the nominal value are displayed. If „Prerun“ is still displayed after starting the measurement, a prerun time is defined in the parameters. This prerun time is used to calm down the test cell which could give higher values due to the exposure to daylight.
After the prerun time the measurement starts automatically.



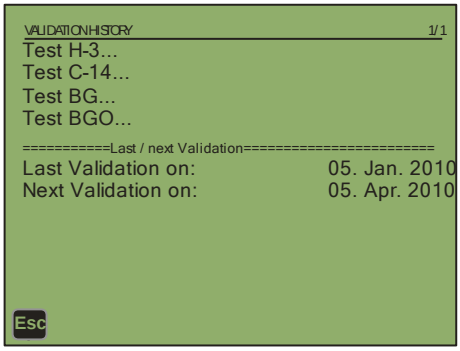
Once the measurement is finished either „PASSED“ or FAILED“ is displayed, depending if the results are within or outside the specified tolerance level.



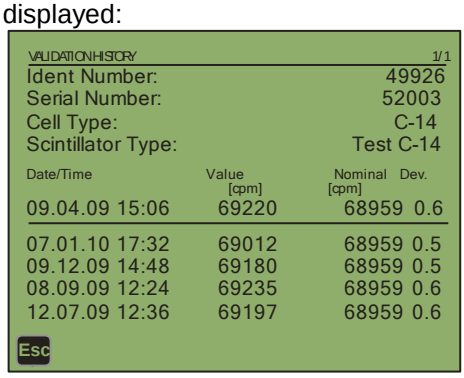
In case of „FAILED“ the measurement can be repaeted by pressing **<Start>.** If the validation is „Passed“ the results can be saved pressing the **<Save>** button. A failed validation cannot be saved.

History

Pressing **<History>** from the validation menu will lead to the test cell selection.



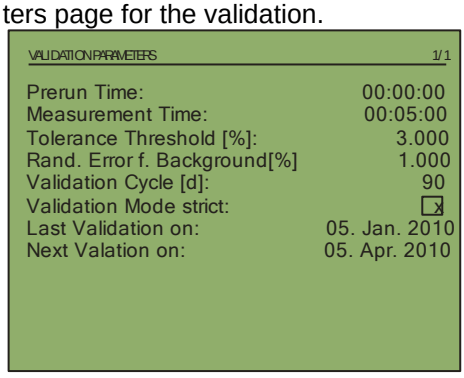
The desired cell can be selected to display their results. After selecting the cell the cell parameters as well as the previously generated results are displayed:



The date of the validation, the measurement value, the nominal value at this date (half live corrected) and the deviation from the nominal value is shown in the table. The entry above the line show the values from the initial validation.

Parameters

Pressing **<Parameters>** from the validation menu will lead to the parameters page for the validation.



- **Prerun Time**
  Defines the waiting time before starting the validation measurement. This prerun time is used to calm down the test cell which

could give higher values due to the exposure to daylight. After the prerun time the measurement starts automatically.

- **Measurement Time**
  Defines the measuring time for the validation (format hh:mm:ss).
- **Tolerance Threshold**
  This is the threshold in % (+/-) which defines if a validation is passed. If the variation of the measured value and the nominal value is outside of the tolerance threshold the validation is failed.
- **Rand. Error f. Background**
  When running a background validation the statistical error is an important criteria. If the statistical error of a validation measurement is higher than specified, the validation is failed although. Statistical errors around 1% at background measurements require a measuring time of at least 1 hour.
- **Validation Cycle**
  Defines the time between two validations. Every successful validation changes the date for the next validation accordingly by the defined amount of days.
- **Validation Mode strict**
  When the box is checked the instrument gives an error message when the validation is due. The instrument cannot be used for measurements.
  If the box is unchecked only a status message appears („Validation is due“ and the instrument can be used.
- **Last Validation**
  Shows the date oft he last successful validation.
- **Next Validation**
  Shows the date when the next validation should be performed. The date can be modified since every validation (e.g. ^{3}H and ^{14}C) increases the date which could lead to a wrong date.

Measured value

The individual test sources may differ from each other up to ±10%. Therefore, only the results from the same test source are comparable. Write down the number of the test source. When using a test source with a different serial number than the previously used one, an error message occurs.

The averaged count rate is used. The nominal values are corrected for the half live automatically.
Please note that results are comparable only when

a) using the same test source,
b) the test source is not being modified (dirt etc.).

Test measurements should be performed continuously and regularly (e.g. once a year). In case of permanent validatiuon failure, please contact your local service department.

7. Startup of the HPLC System

7.1 Preparations

1. Check shipment
   Unpack the instruments and using the enclosed list of parts check if the shipment is complete; then check if the instruments show any sign of damage. The careful packing virtually rules out any damage due to transportation. If you detect any damage to the instruments anyway, please notify the shipping agent, the manufacturer or your local representative.

2. Prepare workplace
   Make sure you have enough space for the instruments:
   The entire measuring system should be set up in such a manner that the capillary connections can be kept to a minimum length. This is especially true for the *column*, the *UV detectors* and the *HPLC Monitor* ***FlowStar²***. Figure 15 shows an example of a possible setup. The capillary connections are identified by straight (_____) lines, whereas the electrical connections are drawn as dotted lines (……).

   To ensure a sufficient air flow through the fan a minimum distance of 10cm must be kept from the rear panel to any wall or instrument. The bottom place of the instrument must be flat to allow the inlet air to flow in. The inlet is located at the bottom of the instrument. Do not cover in- and outlet of the fan.

3. Check power supply
   Check if the power supply indicated on the instrument (rear panel of monitor) matches the local mains supply. If not, do not connect the instrument!

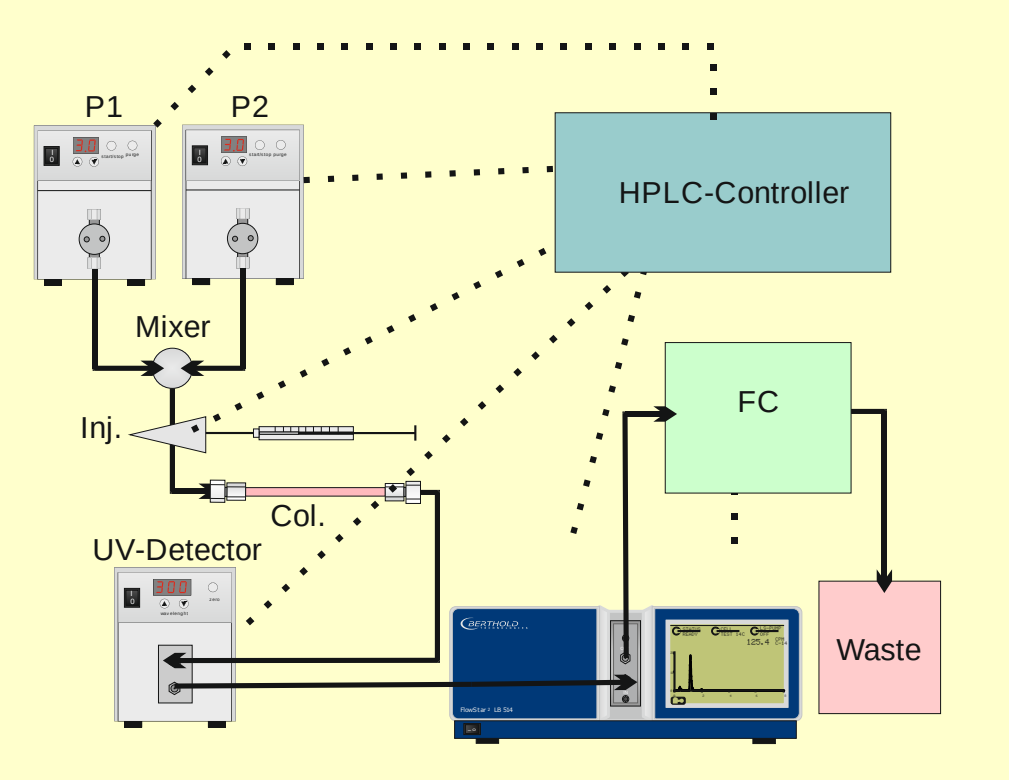


Figure 15: Configuration with solid cell

We have selected the following setup for illustration:

Two eluates are supplied via two pumps (P1 and P2) and mixed. The sample is injected by an injection valve (Inj.). In the column, the sample is separated into individual components (peaks), passed through the UV detector and then to the cell in the HPLC monitor and measured. The eluate flow is split via the 2/3-way valve (V) and the radioactive peaks are collected in the vials of the fraction collector (FC).

- - - - - - = connections for commands and data transfer (control lines)

—=———capillary supply lines for eluate, sample and scintillator

7.2 Electrical Connections

The numbered cables (LB ...) are included in the shipment.

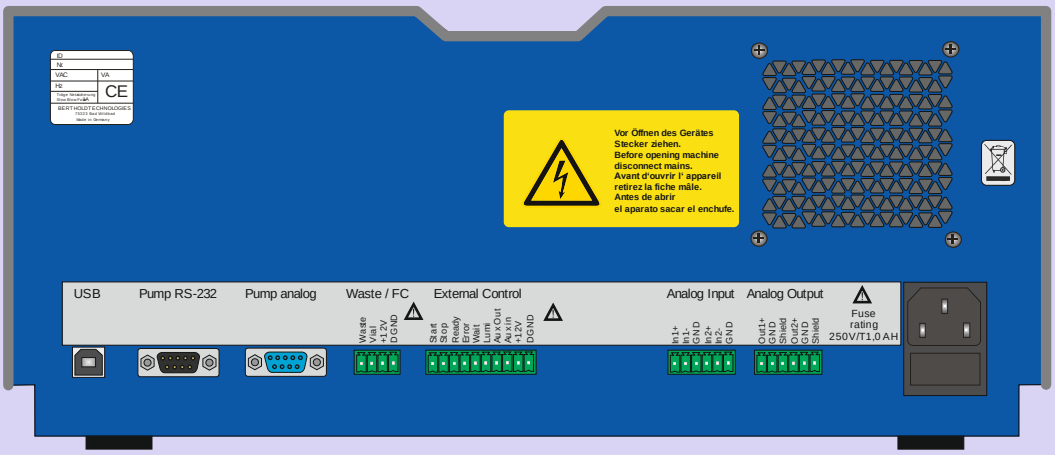
Connect the measuring system to **one** power phase. Connect the entire measuring configuration including all units (such as: gradient controller, UV or other detectors with connected evaluation units and computer) to **one** power phase; otherwise, trouble may occur in the microprocessor units. Use a multipole connector which is connected to the wall outlet.

Mains connections:

1.	***FlowStar²*** *(Main)*	→	cable no. LB 75800-25	→	Mains
2.	***FlowStar²*** (Ratemeter)	→	cable with open ends	→	Integrator
3.	***FlowStar²*** (Ext. Control)	→	cable with open ends	→	Ext. control
4.	***FlowStar²*** (Pump ser.)	→	cable no. ID 26204*	→	Scint. pump
5.	***FlowStar²*** (USB)	→	USB cable A-B	→	Computer
6.	***FlowStar²*** (Waste/FC)	→	cable with open ends	→	Fraction collector

*) The cables marked by an asterisk (*) are not included with the shipment if no pump is ordered. They are part of the pump acceesories.

Terminal Connections FlowStar 2 LB 514



USB
USB PC Connection

LS-Pump ser:
LB 5037 pump control (serial)

Scint. Pump:
LB 5035-3 control (analog)

Waste / FC
Waste: Output Waste valve (OC)
Vial: Vial Change Signal
+12V: Supply Voltage (max. 100mA)
Dgnd: Digital Ground

Ext Control
Start: Start Signal (Input)
Stop: Stop Signal (Input)
Ready: Ready Signal (Output)
Error: Error Signal (Output)
Wait: Waiting for Start (Output)
Lumi: High Luminescence (Output)
Aux Out: Aux-Signal (Output)
Aux In: Aux-Signal (Intput)
+12V: Supply Voltage (max. 100mA)
DGND : Digital Ground

Analog In 1
In1+: Pos. Input
In1-: Neg. Input
GND: Analog Ground
In2+: Pos. Input
In2-: Neg. Input
GND: Analog Ground

Analog Output:
Out1+: Analog Output Channel1
GND: Analog Ground
Shield: Shielding
Out2+: Analog Output Channel1
GND: Analog Ground
Shield: Shielding

Figure 16: Connections on the rear panel of ***FlowStar²*** *with terminal connections*

7.3 Installation of the Measuring Cell

7. Remove the counting chamber transport protection plate on the front panel of the HPLC Monitor which is secured by two Phillips screws (M4, 16 mm long).

8. Take the measuring cell you need out of the protection bag and insert it into the module slot. Check that the O-ring seal sits properly in the groove to ensure that the measuring chamber is protected against light. The ID chip of the measuring cell must face up.

9. Fix the measuring cell module with 2 of the 6 supplied socket head cap screws (M4, 16 mm long). You find these screws, as well as the respective screw driver, in the accessory plastic bag.

   Use only 4 x 16 mm socket head cap screws!

   Fix the screws only slightly first and then tighten them alternatively. Always fix the bottom screw first, since the ID chip on the upper edge of the cell automatically activates the high voltage for the for the light-sensitive detector (if the HPLC Monitor is turned on).

Figure 17: Front view of Radioactivity Monitor ***FlowStar²***

1	=	built-in measuring cell
2	=	fastening screw
3	=	inlet capillary (IN)
4	=	outlet capillary (OUT)
5	=	ON/OFF switch of monitor

7.4 Installation of the Capillaries at the Measuring Cell

13. Use only steel capillaries for supply (IN) and disposal (OUT) of the eluate. Select suitable capillaries for your configuration (supplied or your own).

				supplied:
OUT	solid cell	min. ID	0.5 mm	0.5 mm
	admixture cell	min. ID	1.0 mm	1.0 mm
IN	solid cell	max. ID	0.5 mm	0.5 mm
	admixture cell	ID	1.0 mm	1.0 mm
	(T-mixer -->	measuring cell firmly connected to T-mixer)		

The internal diameter of the outlet capillary must be for

Solid cells ≥ 0.5 mm and for

admixture cells 1 mm.

14. Cut the in- and outlet capillaries to the proper length: by using either a capillary cutter or a feather-edge file to slightly scratch the capillary and then break it – never pinch it off! Check if the bore-hole of the capillaries is open and the connection sleeves of the measuring cells are clean.

15. Install the outlet and inlet capillaries (using either a so-called Swagelock screw fitting or the fingertight screw fitting) onto the connection piece of the measuring cell. For the admixture method, please use the capillary fixed to the T-mixer; the capillary is already bent in a circle to keep light out of the measuring cell.

Installation procedure for finger tight screw fitting (***Figure 20***)

Figure 18: Capillary with PEEK "fingertight" screw fitting

- ❑ Push capillary through the fingertight screw until a piece of it protrudes at the other end.
- ❑ Set screw onto the connecting piece and tighten it slightly.
- ❑ Push capillary all the way into the screw and the connecting piece.
- ❑ Fingertighten screw, make sure it is tight and if you detect any leakage, tighten screw a bit more.

16. Depending on your system setup, connect the outlet capillary to the waste container or – when using a waste valve or fraction collector – to the 2/3-way valve.

7.5 Installation of Other Capillaries

When using other monitors (e.g. UV monitors), the Radioactivity Monitor should always be the last link in the chain**.**

17. Only with solid cells:
Connect the inlet capillaries as required by your measuring setup to the column or other monitors, respectively.

18. Only for admixture cells: (see also Figure 15)
a) Take the scintillator pump LB 5035 into operation: Connect the capillaries and supply the scintillator as described in chapter 0, page 38 et seqq.
b) Connect the capillary coming from the scintillator pump to the T-mixer: Open screw on T-mixer and take out jam cone, then push capillary through screw and jam cone, with the screw thread and the tapering end of the jam cone facing the T-mixer. Push capillary through so that it projects by about 2 - 3 mm on the other end, insert it into the T-mixer and fasten the screw. Tighten the screw fitting only so much that the connection is leak-proof.
In the same manner, fix the capillary coming from the column, the UV detector or the Analytic Splitter (AS xx) to the other inlet of the T-mixer.
c) Installation of the Analytic Splitter: see Chapter 5.2, page 46.

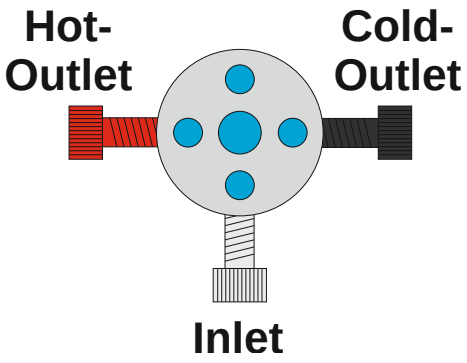
Note:
With short and open capillaries or when using plastic capillaries, a stainless steel capillary of at least 10 cm length should be used between cell connection and open end. Wind the capillary piece around an object of ca. 10 mm ∅ (e.g. ballpoint pen) two times or 4 – 5 times bend in an angle to protect it against incident light.

7.6 Waste valve installation

Electrical connection
The FlowStar² uses the (open collector) output labeled “Waste” to switch the waste valve from ‘cold’ to ‘hot’ Position.

The two waste valve wires will be connected to the “Waste” pin and the “+12V” pin with the supply voltage.

Tubing connection
The valve has 3 tubing connectors. One is the inlet and the 2 others are the outlets. The inlet is not color labeled.



The outlet with the black color label is the so called “cold outlet”. This outlet is active if the status is on non radioactive waste. It must be connected to the waste container collecting the non radioactive waste using the tube with the black fitting.
The outlet with the red color label is the hot outlet. This one is active if the counting level is above the defined threshold. It must be connected to the waste container collecting the radioactive waste using the tube with the red fitting.

Do not connect the clear tube from the inlet directly to the FlowStar² outlet since this will dramatically increase the background due to incoming light.

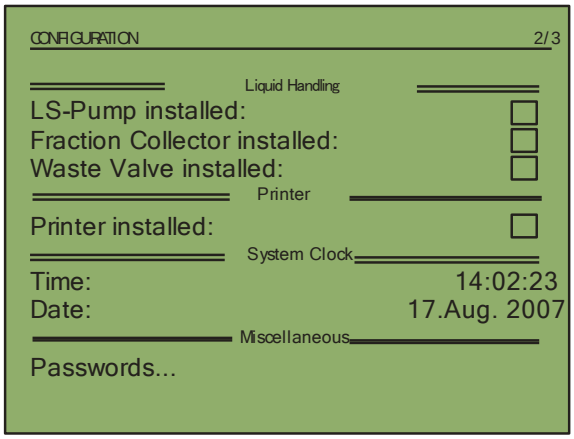
Only use tube unions with a large bore (0.75mm).

Parameter definition

After installing the waste valve the device has to be activated in the FlowStar² hardware configuration.

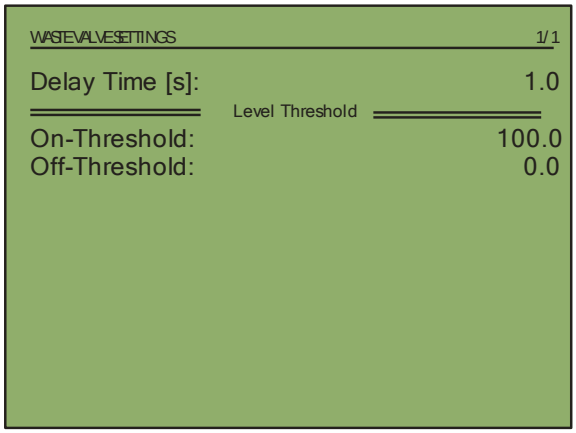
When logged in as system administrator, select INSTRUMENT -> CONFIGURATION from the main menu.

In the second page of the instrument configuration tick the check-box at “Waste valve installed” to activate the valve.



In the measurement method you can now individually activate or disable the waste valve.

A delay time, switch on and switch off level can be defined within each method.



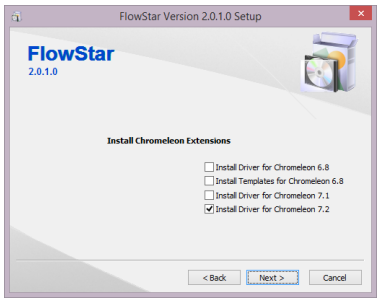
The delay time, from detecting the peak until this fraction reached the valve outside, must be calculated (tube diameter and flow rate required) or measured e.g. with an air bubble as ‘position indicator’.

7.7 Software Installation

To control instrument features of the FlowStar² trough a PC a controller software is required. This software comes with the instrument and allows complete remote control of the instrument as well as test functions.
The software is designed for Windows 7, 8 and 10 (32 and 64 Bit) systems.

To install the software launch the file setup.exe located on the installation media (CD of USB stick). A welcome screen will show up.

Click on next to get to the next screen.



In these dialog box you can select what kind of extensions will be installed.
The USB device driver is essential to communicate to the instrument and should not be disabled. Select the Chromeleon™ feature only, when you intend to control the FlowStar² using Chromeleon® software. Select the correct version of your Chromeleon® installation in order to install the drivers properly. Instrument templates are only available for 6.x versions.
For general purpose control or RadioStar just the FlowStar² driver is required.
The installation starts after clicking on <next>.
After the installation is finished a summary page will appear.

Since the controller uses the TCP/IP protocol for communication a firewall warning message will show up when the controller starts up the first time. To guarantee a proper function of the controller communication must be allowed. If there will be no permission the controller cannot work properly. In this case the windows firewall must be set to allow “flwCtrl.exe” TCP/IP access.

When connecting the FlowStar² to the PC the first time a message will show up that a new device was found. In the upcoming dialog box select “automatic´installation” to install the drivers who have been copied by the FlowStar² installation software before.

Please make sure that the driver was installed before connecting the instrument to the PC to avoid problems with the driver installation.

8. Structure and Operation of the *FlowStar²* Software

HPLC measurements are controlled and evaluated by the ***FlowStar²*** software and presented on the display. The software is operated via the **touchscreen monitor** on the front panel of the ***FlowStar²*** device.

8.1 Power On

Push the toggle switch on the front panel to power up the ***FlowStar²***. A message appears on the display indicating that the parameters are being loaded: the parameters of the currently used measuring cell and the method parameters used last.
Then the **Measurement** menu is displayed together with the curve diagrams of both counting channels. The device goes directly to the Rateme-ter mode and displays the measured values as a curve.
In addition to the 3 **info buttons** in the top section of the display which are used to view the parameters, you can only select the function key for password entry.
The other options are available for selection only after you have logged on as an authorized user or device administrator and entered your password.

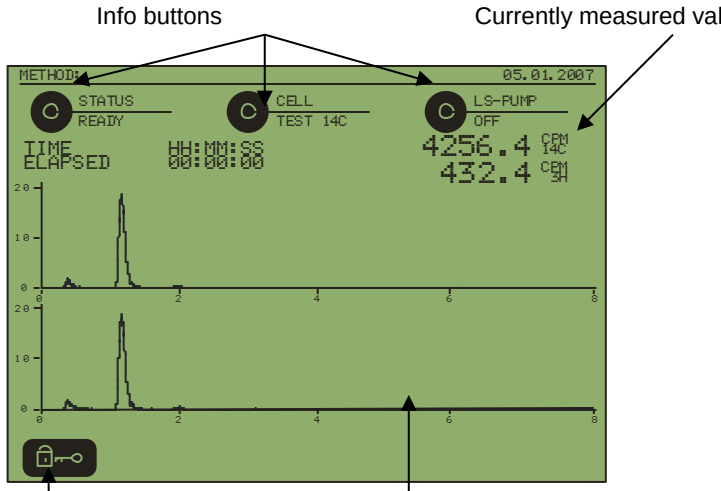


Figure 19: Measurement menu after power on

8.2 Operation

The software is operated via the **touchscreen monitor**. A **touchscreen monitor** is screen and keyboard at the same time:
To select a function or an item, push with a suitable pin (or your finger) on a function key, menu item or a parameter row.

Function keys

Function keys are depicted as black buttons with a corresponding caption (e.g. **Start**, **Menu**), a symbol or an abbreviation (**Esc**).
The caption of **info buttons** appears next to the button and is connected with the button by a line (e.g. the **Status** info button on the **Measurement menu**, see Figure 19).
Push a function key to execute the function.

The following function keys are used:

Leads to the password entry (user login).

Esc

Takes you back to the previous program level. Push this button, for example, to go from the **Main menu** to the **Measurement menu** (see Figure 19) or from a parameter page back to the **Main menu**. Possible changes are taken over and stored.

Menu

Takes you from the **Measurement menu** to the **Main menu**. This button is not displayed during a run.

Start Stop

Starts or stops a measurement.

Pg

Scrolls through the measurement menu to the next page.

X

Push this button to move the measurement image back by one display width. Thus, you can scroll back in the measurement display. This is possible max. 3 times. If you push this button for the 4th time, the measurement image returns to the online display.

The select bar jumps to the top or bottom row of the respective parameter page. If you are already there, you go to the previous or next page (if there is one).

Every time you push an arrow button, the select bar moves to the next or previous row. Parameters that cannot be edited will be skipped.

The **Enter** button has two different functions:
In a parameter list: To edit a parameter: A new page is displayed for selection of defaulted parameters or to enter alphanumeric values.
For alphanumeric input: To confirm and accept entries.

Highlighting a row
To highlight a row, either push this row or repeatedly push the function key ↑ or ↓.

Selecting parameters
To select a parameter from a selection list you have to highlighting this list first and then push the function key ↵.

Alphanumeric entries
To enter alphanumeric characters, push the buttons on the keyboard displayed on the monitor.

↑ shift key;
<Del> deletes the last letter;
↵ accepts the entry.

Selecting menus and submenus

- Push Menu to go from the **Measurement menu** to the **Main menu**.
- To select a menu on the **Main menu**, push the button with the respective menu name. This takes you to a parameter page or to a further branch.
- On a parameter page, a submenu is identified by 3 dots next to the submenu name: e.g. **Display configuration...** in the following illustration. To select the submenu, highlight it and then push the ↵ key.

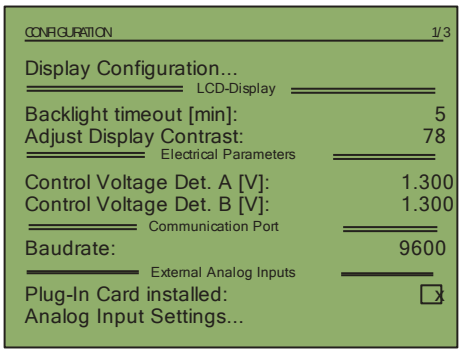


*Figure 20: Parameter page with submenus (e.g. **Display configuration...**)*

Info buttons (see also chapter 15. Troubleshooting Check List)

<Status> button
Shows the general system status. Push the <**Status**> button to go to the status page showing more information on the current system status, e.g. which events have occurred.

We distinguish 5 states:
Ready, **Error**, **Warning**, **Info** and **Measurement**.
Error, **Warning** and **Info**: the respective text (below the line to the right of the button) is displayed flashing inverse.
Warning and **Error**: there is an additional acoustic signal.

Ready display — The device is ready for measurement, no error/warning/info messages are displayed.

Error display — An error has been detected, the measurement was stopped completely.

Error events:

- Leakage
- Counting chamber open
- Detector A+B HV off
- Cell chip error
- Detector error

Warning display — A warning event has occurred, no errors have occurred.

Warnings:

- High luminescence
- Overflow det. A
- Overflow det. B

Info display — An info event has occurred, no errors / warnings have occurred.

Infos:

- System test overdue etc.

Measurement display — A chromatogram measurement is active, no error/warning/info messages are displayed.

<Cell> button

Regular operation
During regular operation, the current cell type is displayed as text next to the button. Push the button to view the cell parameters.

In case of error
If an error has occurred while reading the cell or if the counting chamber is open, then the text **Error** is displayed next to the button flashing inverse.
If you push the button now, you do not go to the cell parameters; instead, a page comes up showing more information on the error that has occurred.

<LS pump> button
This button is displayed only if an LS pump is connected and the respective parameters have been entered.

Regular operation
During regular operation, the current status of the LS pump is displayed next to the button: on or off. Push the button to view the pump parameters.

In case of error
If an error has occurred in the pump control, then the text **Error** is displayed next to the button flashing inverse.
If you push the button, a page comes up showing more information on the error that has occurred.

8.3 Password Entry

Before you can work with the system, you have to log-on with your user status so that the system knows which rights the respective user has. There are two different access levels:

User This type of user does not need a password (default setting). He or she cannot change any parameters, but only start/stop measurements, etc. Not all menu items are displayed. On request, a password may be set for this user.

Device administrator This type of user can define methods and edit all required measurement parameters. All menu items except for the service specific functions are displayed. A password is required (factory setting „0“).

Proceed as follows:

- ❑ On the **Measurement menu**, push the button. A new window appears showing the types of users that are available for selection.
- ❑ To select a user type, highlight the desired type and select it with the button.
- ❑ If you have selected **User**, the program returns to the **Measurement menu** and shows further function keys for operation.
- ❑ If you have selected **Device administrator**, a complete alphanumeric keyboard is displayed on the screen so you can enter the password.
- ❑ Enter your password and confirm it with . The program returns to the **Measurement menu** and shows further function keys for operation.

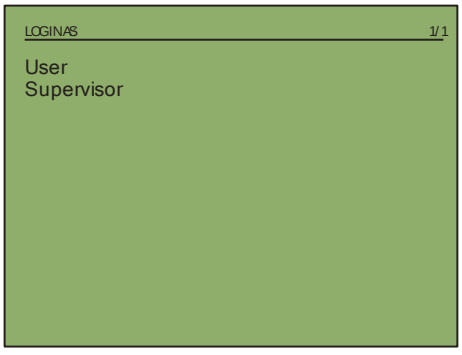
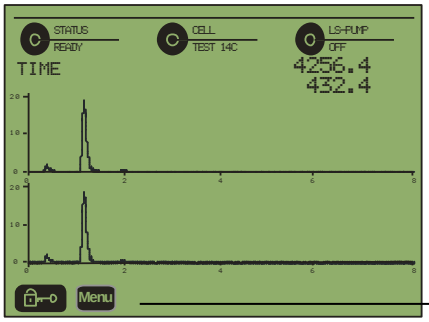


Figure 21: Selection of the type of user

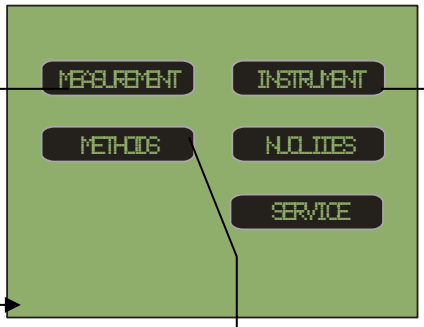
***Note:* If the device is not operated for 10 minutes (i.e. no function is executed via the touchscreen monitor), the user is automatically logged off.** This ensures that unauthorized persons do not have access (for example, if the logged-on user has to leave the room).

8.4 Menu and Function Overview

Measurement menu

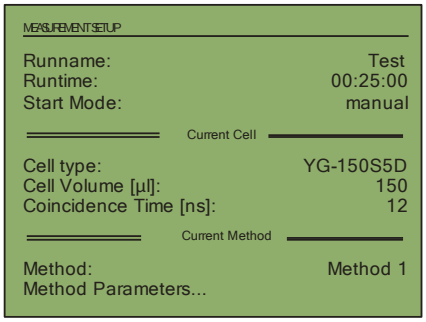


Main menu

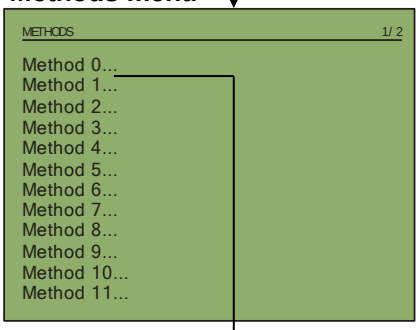


See next page

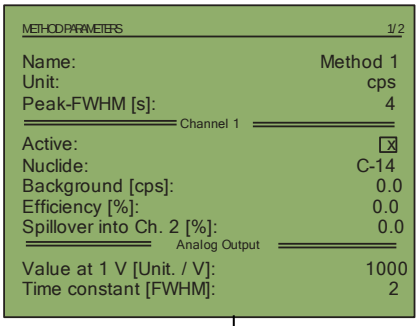
Measurement menu

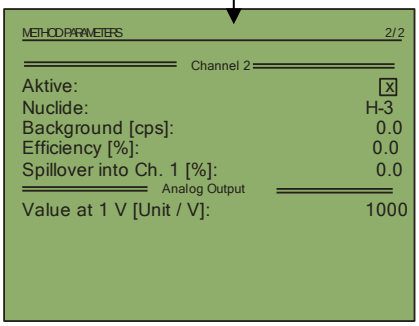


Methods menu



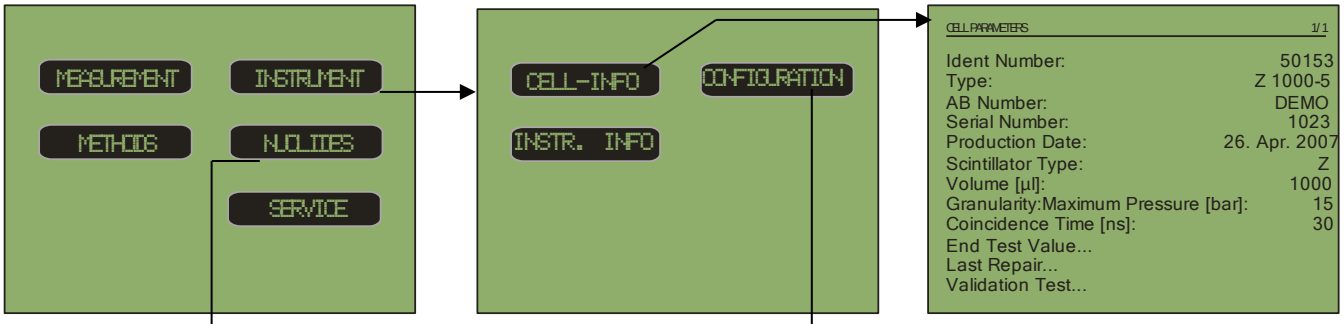
Individual methods



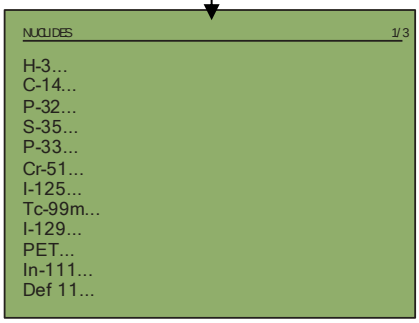


Configuration menus

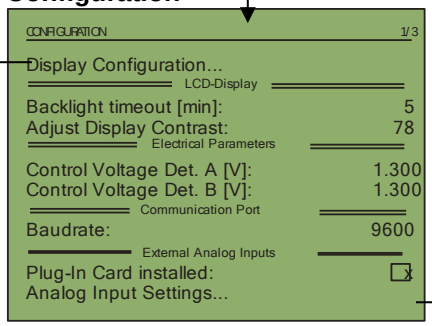
Main menu

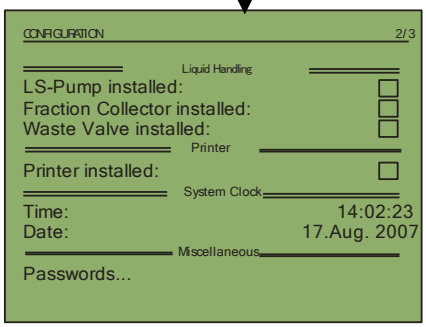


Nuclides

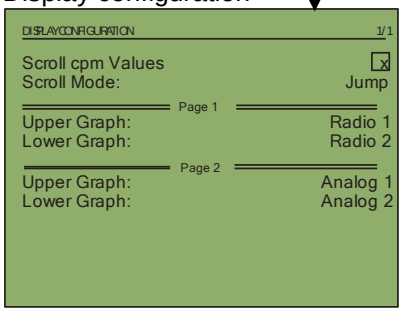


Configuration

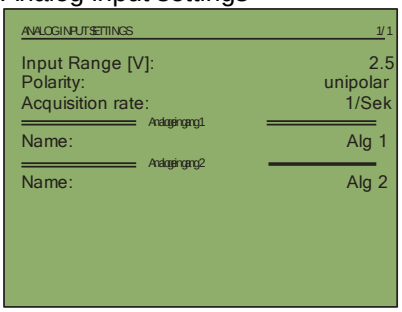




Display configuration



Analog input settings



9. Parameter Entries

The parameter entries for the individual methods depend on the configuration of the device and the measuring cells used. Based on the configuration and the measuring cell used, parameters are automatically defaulted and only the relevant information is displayed, so that methods can be defined easily and safely.
We will describe the parameter entries in the following order:

- Configuration of the device (with connected devices)
- Nuclide parameters (with threshold values and permitted measuring cells)
- Measuring cell parameters (automatically defined by the cell that has been inserted)
- Definition / Selection of methods
- Definition of measurement parameters (parameters for one run)

Once the basic configuration of the device has been set up and the methods have been defined, all you have to do to start a measurement is select a measurement method and define the measurement parameters (duration of measurement) for the current run.

9.1 Configuration of the Device

The device configuration is defined on the **Configuration** submenu.

❑ On the measurement menu, select **Menu | Device | Configuration**. The first of three parameter pages is displayed.

Configuration parameter page 1 / 3

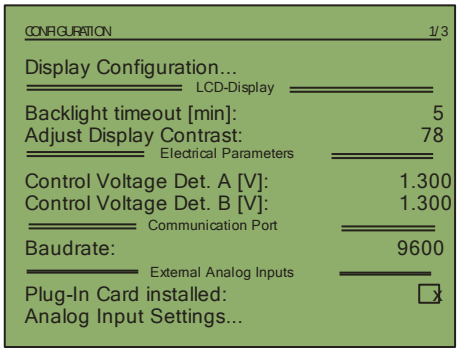
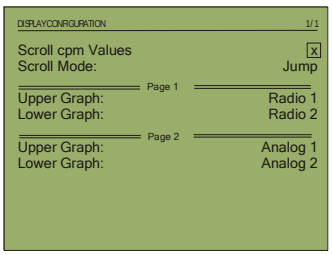


*Figure 22: **Configuration**, parameter page 1/3*

Display Configuration...

Here you can define which counting channels are to be displayed and how the measurement diagrams should be arranged on the **Measurement menu**.



❑ Select **Display Configuration...**, and push the ↵ button to go to the parameter input.

❑ Define on which page the radioactivity signals and where channel 1 and channel 2 are to be displayed.

❑ Highlight **1st page | Top Graphic**, and push ↵.

❑ In the following selection list (**Top Graphic**), select the channel that is to be displayed on the first screen page at the top. You may select both analog channels **Analog1** and **Analog2** and both radioactivity channels **Radio1** and **Radio2** and also the item **None**.

❑ Proceed in the same manner with the other three screen positions.

❑ Define the scroll mode for the image display. You may choose **Jump** and **Scroll**.

Jump: If the measurement curve has reached the right edge of the display, the measurement image jumps to the left by half a display width.

Scroll: The measurement image scrolls continuously to the left. If the graphic display starts to flicker, change to **Jump**.

LCD Display

Illumination off after [min] Here you can define after how many minutes the screen illumination is to be turned off.

Adjust display contrast Here you set contrast and brightness of the screen. Values between 73 (bright) and 85 (dark) are possible.

Electrical parameters

Control voltage Information about the control voltage for detector A and B. These parameters are only accessable through service personal.

Data comm. interface

Baudrate The baudrate is 9600.

External analog inputs

Plug-in card installed Tick the checkbox (x) if you have installed a plug-in card for analog inputs. To enable/disable this item, highlight it and then push the ↵ button.

Analog input settings... If a plug-in card is installed, you have to define the following parameters:

Analog input settings...

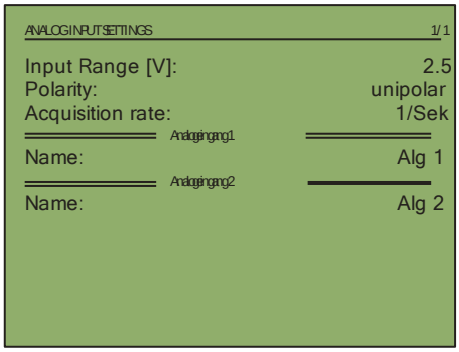


Figure 23: Analog input settings

Input range [V] Select the defaulted ranges: 0,156, 1.25 or 2.5

Polarity Select the polarity (unipolar/bipolar)

Acquisitions rate Select the scan rate: 1/sec, 2/sec, 4/sec or 8/sec

Analog input 1 / 2

Name Enter the name of both analog channels. This name appears in the measurement diagrams.

Parameter page Configuration page 2/3

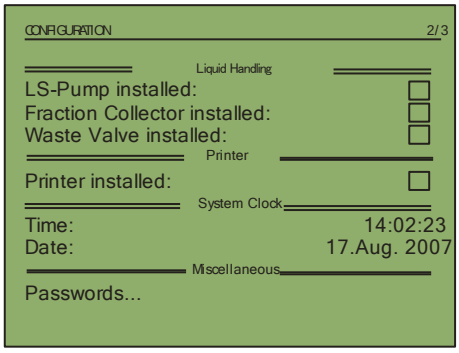


*Figure 24: **Configuration**, parameter page 2/3*

Liquid handling

LS-pump installed Tick the checkbox (x) if you have are working with an LS pump. The respective queries will appear in the methods parameter dialog only if this checkbox is ticked. Only possible with admixture cells.

LS Pump Settings… Select the correct pump to setup the pump communication. “Analog” will use the analog output signal at the “Scint Pump connector to control the flow rate. All other settings will control the pump through the serial port at The CPU. LB5035 is for older pumps, the LB5035-3M is the microbore version (reduced flow rate). LB5036/5037 represents the current pump version.

Vol. Cal. Factor This Factor can be used to calibrate the pump to a specific scintillator. Due to different viscosity of liquid scintillators the real flow rate can differ from the preset flow. Using the calibration facto the flowrate can be corrected to get correct flow rates. Default is 1.000

Count if pump is off If this box is ticked, the FlowStar² will display no counts if the scintillator pump is not running.

Data Output Delay (s) When the previous box is ticked an additional data delay can be set when the pump starts working. This will avoid the display of ghost peaks due to improper mixing right after starting the pump. Useful values are between 10 and 30s, defaiult is 0. If set to 10s the FlowStar² will start displaying data 10s after the pump started.

Fraction collector installed Tick the checkbox (x) if you are working with a fraction collector.

Waste valve installed Tick the checkbox (x) if you have a waste valve installed.

Thresholds

Luminescence warning

Defines the level fort he luminescence warning. The higher the value, the more sensitive the luminescence warning. The luminescence warning is disabled when entering „0“.

Printer

Printer installed This feature is not implemented.

System clock

Time / Date Enter the current date and time.

Parameter page Configuration page 3/3

Others

Passwords Here you can enter one password for the user level and one for the device administrator level.

Test cell installed This setting is for external gamma test sources. If the box is checked the BGO cell is enabled to perform a system validation since external gamma test sources do not have an ID chip.

Language Here you define the language for the user interface: German or English.

9.2 Nuclide Parameters

The ***FlowStar²*** software includes a nuclide table on the **Nuclide** menu which contains the major nuclides for the HPLC methods including half-life period, energy thresholds and the measuring cells that can be used. The parameters of these nuclides cannot be changed by the user.
You can also define your own nuclides or nuclide mixtures with user-specific parameters in the nuclide table.

❑ Select **Menu | Nuclide** on the measurement menu.

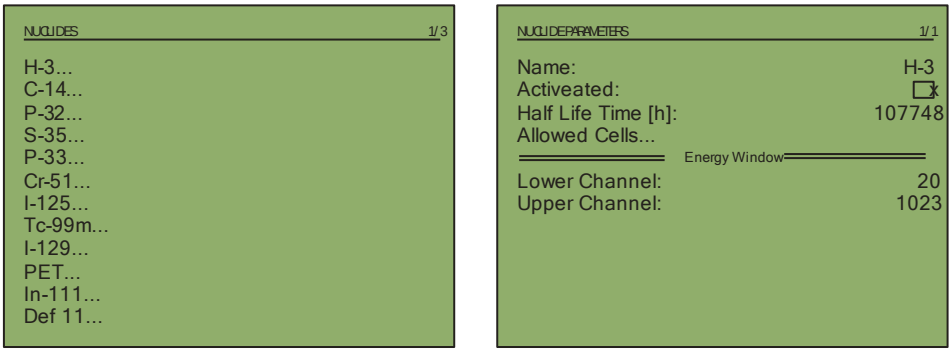


Figure 25: Nuclide table (left); parameters of a selected nuclide (right)

The 1st page of the **nuclide table** lists the supplied nuclides; on the following pages you can define your own nuclides. These have the default name **Def 11**, **Def 12** etc. or **USR-0**, **USR-1** etc.

Editing a user-defined nuclide

Highlight the desired nuclide (Defxx or USR-x), and push the ↵ button. The page with the respective nuclide parameters is displayed.

Name — Enter a name for the nuclide (max. 6 characters).

Enabled — Only if the checkbox of a nuclide is ticked (x), it can be selected for a method.

Half-life [h] — Information on the half-life period in hours.

Permitted cells... — If you select this item, a list of available measuring cells is displayed. Tick the measuring cells (x) that are allowed for the respective nuclide. Thus, the nuclides allowed for a method are already selected when inserting a measuring cell.

Energy window

Gain high/ low — The gain setting for the amplifier must be set here. Depending on the expected signal the gain must be either low (x1) or high (x10).

Low / High channel threshold — Define the energy window for the respective nuclide by entering a high and low threshold. If 2 windows are required for a nuclide, define new nuclides accordingly (see below).

Permitted cells...
Select the measuring cells (x) that are to be used to measure this nuclide. Only those nuclides are offered for selection in the method definition which are permitted for the installed/pre-selected measuring cell.

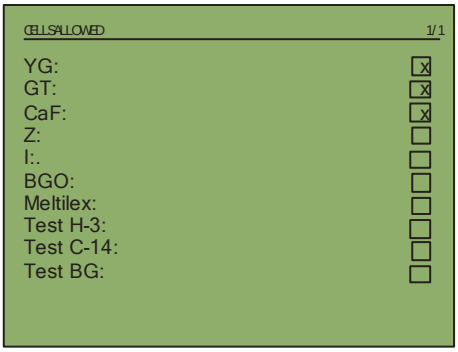


Figure 26: Permitted cells are identified by an x

9.3 Defining / Editing Methods

The parameters required for this configuration are defined and edited on the **Methods** menu depending on the device configuration and the installed measuring cell.

❑ On the measurement menu, select **Menu | Methods**. The **Methods** window is displayed showing a list of 20 possible methods: **Method 0** to **Method 19**. You can define up to 20 different methods.

❑ Highlight the desired method and push the ↵ button to view the **Method parameter**s (two pages).

Method parameters page 1

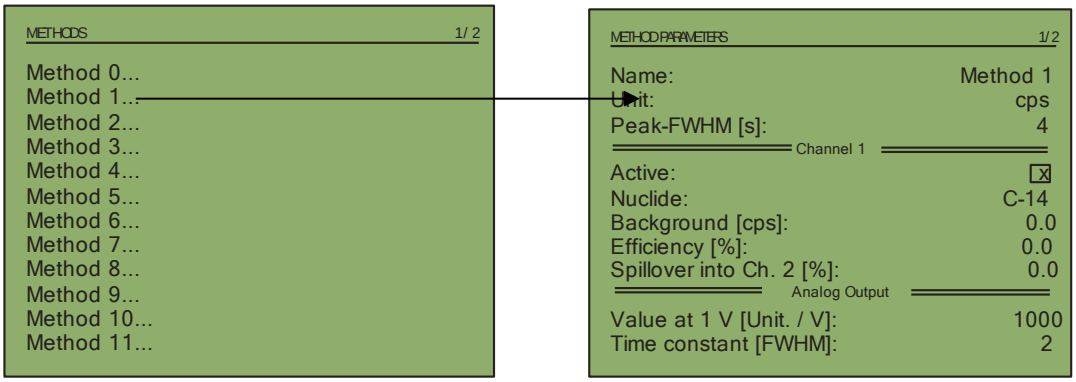


Figure 27: Left: List of methods. Right: Method parameters, page 1

The individual parameters

Name Enter a name for the method.

Unit Select the desired unit. Push the ↵ button to display the selection list with the possible units: cps, cpm, Bq, dps, dpm and the display of the current value.

Peak half-width [s] Select the peak half-width. Push the ↵ button to display the selection list with the possible half-widths. 4; 8; 15; 30; 60 and the current value. The peak half-width is used to adjust the curve smoothing to the expected peak width. If different peak widths exist in a run, you should enter the half-width of the narrowest peak, so that all relevant and possibly existing overlapping peaks will be covered.

A detailed description of the smoothing feature can be found in chapter 13.1

Channel 1

Active Tick the checkbox (x) if you want to enable channel 1.

Nuclide Select the desired nuclide. Push the  button to view the selection list with the possible nuclides. Depending on the measuring cell used, only the nuclides are displayed that are permitted for this measuring cell (see nuclide table).

Background [cps] Enter a background value, if desired. The background has to be determined by a separate measurement. The value entered here is automatically subtracted from the measured value, so that net values will always be output (on the display, the printer and the ratemeter output).

Efficiency [%] Enter the efficiency in %. This value is used for the efficiency correction, if it is enabled.

Spillover into channel 2 [%] This parameter is only important for 2-channel measurements with two different nuclides. Enter the spillover into channel 2. This parameter is used to correct for the spillover of this nuclide into the second channel. This correction is done after subtraction of the background.

Enter **0.0** if you do not want to carry out any spillover correction.

If you measure one nuclide with 2 channels, this parameter is not relevant (see remarks below). In this case you also have to enter **0.0**.

Analog output Settings for the analog output. Smoothed analog signals that are proportional to the measured count rate will be output here. You have to define the measuring range within which the count rate is to be expected.

Value at 1 V [Unit/V] Enter the expected measuring range that corresponds to 1 V. Entering 1000 means that the measuring range is 1000 cps (with the preselected unit cps) and corresponds to 1 V. Values above that can be presented up to about 3V output voltage. If the count rates are within the selected measuring range, analog signals which are proportional to the count rate are output in the range from 0 to 1 Volt. Higher count rates are then output linear up to 3-times this value (3 Volt).

Time constant [FWHM] Via the time constant you define the weighing of the smoothing of the analog signals. The peak half-height width (FWHM) serves as measure for the time constant. The higher the selected time constant, the more accurate even weak peaks will be detected. On the other hand, the peak form will be slightly flattened. Preferably one should use values of 1 and 1.5. If you enter 1, smoothing is performed over the range of one half-life width.

You may choose: 0.5; 1; 1.5 and 2 half-height widths.

A detailed description of the smoothing feature can be found in chapter 13.1

Measurement of <u>one</u> nuclide with two different analog output settings

The measurement of one nuclide in two channels with different windows may be relevant when ***FlowStar²*** is integrated into an HPLC system through an alanlog output signal transfer. This will cause losses in presentation of the result, which are due to the small range of the analog signal (0-1 V) and the dynamic range (2 decades) of the HPLC system. ***FlowStar²***, however, can represent a range of 0-2.5 V at a dynamic range of about 6 decades.
To get a more accurate presentation of smaller signals and peaks, one channel can measure and present the results using a lower analog output setting (e.g. 1.000), the other channel should be set to a high analog output setting (e.g. 100.000 or 1.000.000) to display the higher intensity peaks more precisely and avoid an overflow.

<u>Method parameters page 2</u>

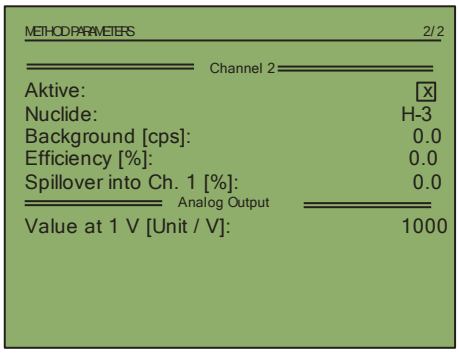


Figure 28: Method parameters page 2

The individual parameters

<u>Channel 2</u> Proceed as with channel 1.

<u>Analog output</u> Proceed as with channel 1.

<u>LS pump</u> Appears only when the LS pump is enabled (see Configuration menu, parameter page 2, chapter 9.1).

Eluate flow [ml/min] Standard flow rate: 1ml/min; with preparative samples: 10 ml/min.

LSP flow [ml/min] Flow rate of scintillator pump (only applies if pump and Z cell are installed)

Start time Enter the start time for the LS pump.

Stop time Enter the stop time for the LS pump.

9.4 Measurement Parameters

The term measurement parameter refers to a group of parameters that are required for a run. They comprise the **measuring cell parameters** (defaulted by the measuring cell that is used), a **measurement method** and the variables **Run time** and **Start mode**. If you have a scintillator pump, a fraction collector and a waste valve connected, the respective parameters also belong the variable measurement parameters.
The measurement method is selected and the variables are defined on the **Measurement** menu.

Define measurement parameters and start run - Proceed as follows:

- ❑ Select **Menu | Measurement** on the measurement menu. The **Measurement parameters** page is displayed.
- ❑ Define the desired parameters:
- ❑ Push Esc to exit this/these page(s). The measurement parameters will be stored. The program returns to the main menu.
- ❑ On the **main menu**, push Esc. The program goes to the **Measurement menu**.
- ❑ If you push Start on the **Measurement menu**, the run will be started with these parameters. In the top row of the **Measurement menu** the method used and the name of the run are displayed alternatingly (as defined in the measurement parameters).

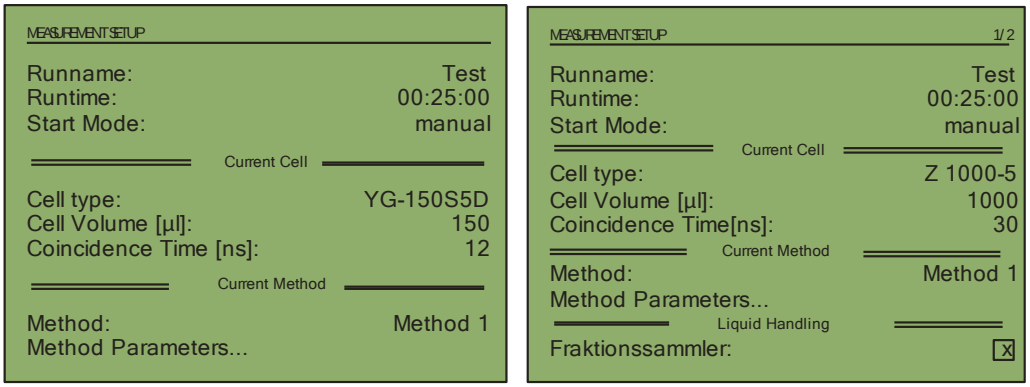


Figure 29: Liquid handling disabled (left) and enabled (right)

Measurement parameters page 1

Run name Enter the name of the run. This name is displayed on the **Measurement** menu while a measurement is running and it also appears on the printout.

Run time Enter the duration of the run (max. 24:59:59).

Start mode You can choose **manual** or **external** as start mode. Manual means that you have to push the function key Start on the **Measurement** menu. If you select **external**, the control takes place via an external device (connect to "Start" at the **External Control** connector block on the device rear panel)

Current cell The parameters for the cell used are displayed here. This entry cannot be edited. If necessary, you have to replace the measuring cell.

Cell volume [µl] Cell volume in µl.

Coincidence time [ns] Is defined by the measuring cell used (decay time of the scintillators): Solid cells: 100 nsec; Liquid cells: 30 nsec.

Current method Select the desired method.

Method XXX Selection and display of the desired method for this run. Push the ↵ button to view the selection list with the methods that are compatible with the measuring cell used. If no method list is displayed, no method has yet been defined for this measuring cell.

Method parameters... Push the ↵ button to display the parameters of the selected method. At this point, the method parameters cannot be edited.

Liquid handling These parameters will be displayed only if the LS pump has been enabled on the menu **Configuration | Device**. In this case the measurement parameters cover 2 pages.

Fraction collector Tick this checkbox if a fraction collector is employed.

Measurement parameters page 2

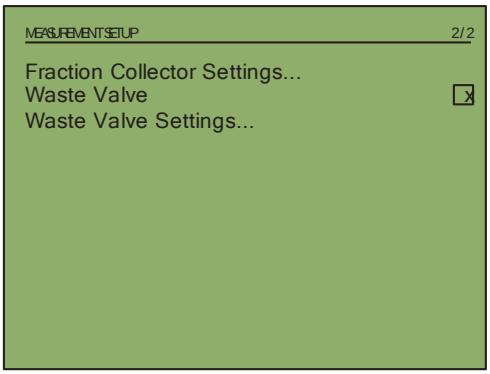


Figure 30: Measurement parameters page 2

Fraction collector settings... Is displayed only if the item **Fraction collector** is enabled Upon selection of this item you go to the page **Fraction collector settings** (Figure 31).

Waste valve Tick this checkbox if a waste valve is employed.

Waste valve settings... Is displayed only if the item **Waste valve** is enabled. Upon selection of this item you go to the page **Waste valve parameters** (Figure 32).

Fraction collector settings

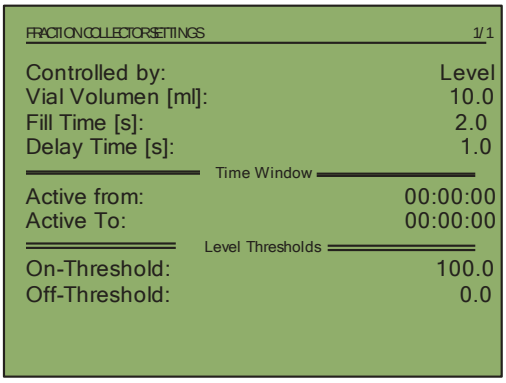


Figure 31: Fraction collector settings

Controlled by Control of the fraction collector either via **Threshold** or **Interval**.

Vial volume [ml] Defines the vial volume.

Fill time [s] Fill time for fractioning into vials (with broad peaks that have to be filled into several vials). It is calculated by the system based on the vial volume and the flow speed.

Delay time [s] Time delay from the cell output to the fraction collector.

Time window

Active from ... to ... Enter the time for enabling the fraction collector.

Threshold value Only if you have selected **Threshold** in the **controlled by** field. Enter the low and high threshold value.

Interval Only if you have selected **Threshold** in the **controlled by** field. Enter the interval time in seconds.

Waste valve parameters

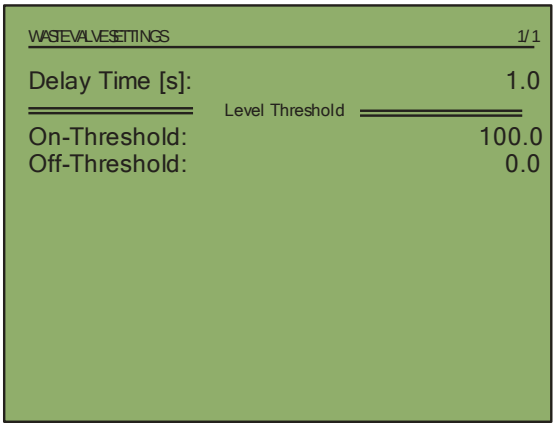


Figure 32: Waste valve parameter

Delay time [s] Time delay from the cell output to the waste valve. This time delay depents on the specific flow rate and the length of the tubing used and must be tested individually.

Threshold value Enter the low and high threshold value for the waste valve control. Please keep in mind that the switch-off threshold must be smaller than the switch-on threshold.

10. Measurement

The device includes two measurement modes:

- Continuous measurement: A continuous measurement starts automatically after the device has been powered up using the measurement parameters defined last (chapter 9.4). If you define parameters, for example, the measurement continues to run in the background. The measurement in the background is stopped temporarily only when the energy spectrum is called in the service functions. This mode is useful when no external hardware is controlled through the FlowStar² since this requires an external start signal.
- Measurements over a limited time: After selection of a method and definition of the measurement parameters, these measurements are started on the **Measurement** menu and can be stopped again. This measurement mode must be used when external hardware (scintillator pump, waste valve etc.) is controlled though the FlowStar². It requires either a manual start (touch screen) or an external start signal.

10.1 Measurement Menu

The currently measured values of both counting channels are displayed graphically and numerically on the **Measurement** menu. Push Pg to switch between display of the radioactivity and the analog signals.

Info row and info buttons

First row The method and the run name are displayed alternatingly.

<Status> Shows the current status. Push this button to view possible error messages (see chapter 8.2 and 15).

<Cell> Shows the currently used type of measuring cell. Push this button to view the measuring cell parameters.

<LS pump> This button is displayed only when the scintillator pump has been enabled on the **Configuration** menu. The pump status is indicated (either **ON** or **OFF**). Push this button to view the pump parameters.

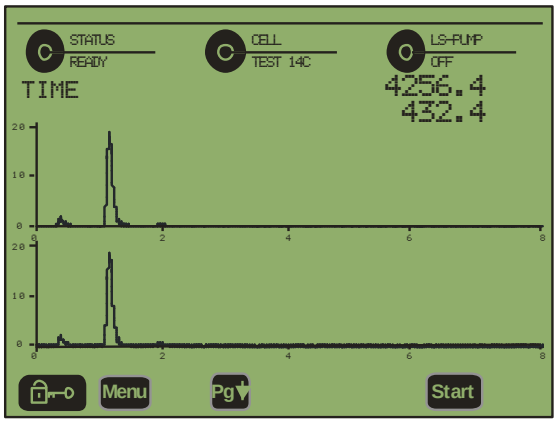


Figure 33: Measurement menu

10.2 Run Measurement

Instructions for starting/stopping a run

- A run can be started any time on the **Measurement** menu by pushing the Start button. The measurement parameters defined last will be used (**Measurement** menu).
- If you have **edited measurement parameters** during a continuous measurement or a measurement over a limited time, these changes are not taken into account. They are used only when the changed parameters (on all levels up to the measurement parameters) are loaded again and the measurement is started again.

  Example: Changed energy window of nuclide XY.

  - ❑ First, open the method in which this nuclide is used and select the nuclide XY again. Only then the nuclide with the new parameters will be taken over into the method.
  - ❑ Select the **Measurement** menu and there select the method again for the respective run. Only then the *changed* method will be loaded.
  - ❑ Now go to the **Measurement** menu and start the measurement. Only then the new parameters will be used, in this case the new energy window of the nuclide.

  This also means that in all methods which use the nuclide XY, the nuclide has to be loaded again so that the new nuclide parameters will be used.
- A run over a defined time period is automatically terminated as soon as this time period is over. You may also stop a run any time by pushing the Stop button.
- At the end of the run, the measured data are stored and are available for printout or for data transfer.
- The data may also be transferred online to the PC and displayed on the screen, e.g. using the ***RadioStar*** software and processed further.
- At the end of the run, the device goes to the continuous measurement mode and shows the measured results on the display. In addition, they are output as analog signals to the analog output.

Proceed as follows to define and start a run:

- ❑ Select the **Measurement menu** on the **Main menu**. The **Measurement parameters** page is displayed.
- ❑ Define the measurement parameters and select the desired measurement method as described in the previous section.
- ❑ Exit the measurement parameters page with Esc. The program returns to the **Main menu**.
- ❑ Push Esc to go to the **Measurement menu**.
- ❑ Push the Start button.

Starting a run (externally)

If **Start mode external** is defined in the measurement parameters (and the necessary connections have been established), the run is started/stopped by an external start/stop signal (instead of pushing the Start button). Everything else is the same.

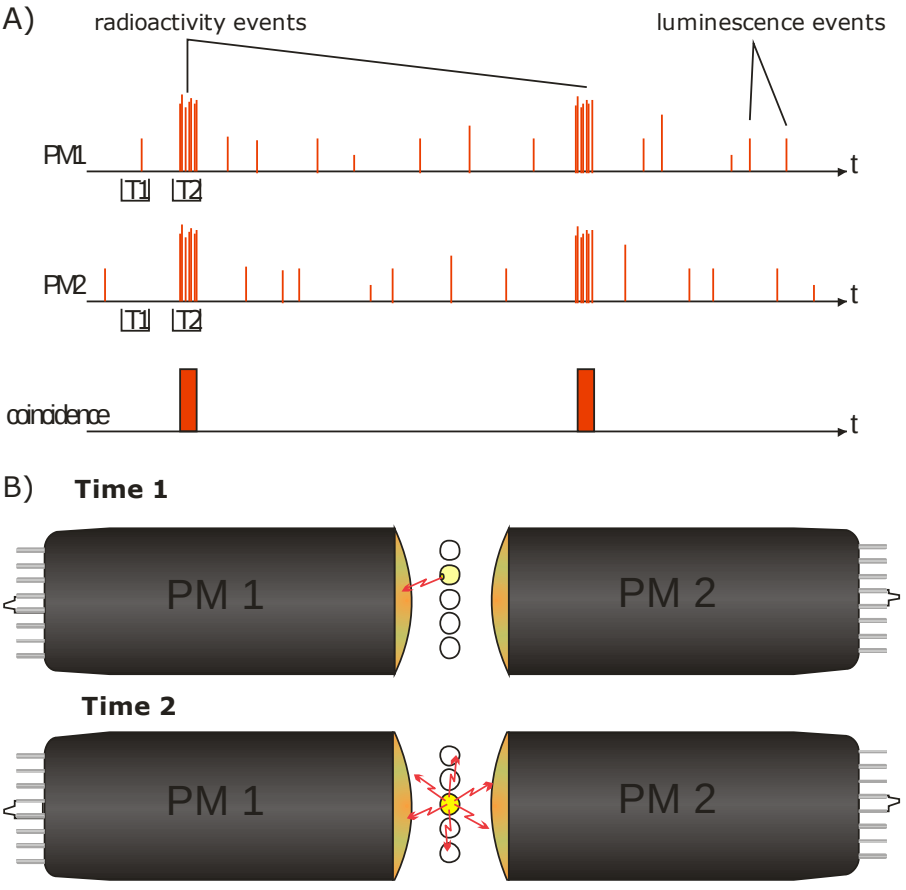
11. Function of Measuring Cells

A number of different Berthold measuring cells are available for radioactivity measurements in column eluates; all common sources can be measured with high efficiency with these cells. Depending on the measurement method and application, the cells have been designed for the respective measurement configuration and, therefore, they possess different properties. They are designed for highest efficiency and can easily be adapted to any flow-through system such as HPLC, LC, FPLC, MPLC, HPLC-MS, SFC.

The Radioactivity HPLC Detector ***FlowStar²*** essentially comprises two components:

- The measuring cells may be regarded as an “interface” between separation system and radioactivity measuring system. Different measuring cells permit adaptation of the radioactivity flow-through monitors to existing chromatographic condition, such as separation or flow-through system, the nuclide to be measured, the activity level to be measured, the resolution and the flow rate. In the measuring cells, the nuclear radiation contained in the sample is converted into luminescence (light) signals through stimulation by a scintillator.
- The light pulses emitted in the measuring cells are registered by the detectors and converted into electrical pulses and analyzed, and then downloaded to a data system for evaluation.

Radioactivity flow-through detectors register radioactivity by means of scintillators or via the Cerenkov effect. The radioactive atoms in the labeled substances disintegrate and excite the scintillator in the measuring cell through adsorption. The scintillator decays in a very short time, emitting photons, a so-called light flash or light pulse. The decay time or the time distribution of the photons generated per light pulse (pulse width) is a specific feature of the scintillator used and lasts for a few to several 10 nanoseconds (1 nanosecond = 10^{-9} sec). The frequency of the generated light pulses (pulse number) is proportional to the number of adsorbed ionizing rays in the scintillator. The luminescence signal generated by the radioactivity differs from chemi- or bioluminescence with regard to the time distribution of the photons. Light that has been generated by chemi- or bioluminescence appears as statistically “homogeneous” single photon events distributed over time, whereas the light signals generated by radioactivity occur as a bundle of several photons which occur in a very short time (around 10^{-8} sec). See also Figure 34.



T1: Time 1; T2: Time 2; PM1: Photomultiplier 1; Pm2: Photomultiplier 2

Figure 34: Illustrates the difference between luminescence and radioactive events

Radioactive events occur in a “bundle”, so that both PM’s register photons (time 2), whereas luminescence events represent single photon emissions.
Only coincident events will be processed as useful signals.
A) Presentation on the time axis
B) Presentation in the measurement chamber with PM 1 and PM 2

The light signals are detected and amplified in the flow-through detector by 2 photomultipliers (PM) located on both sides of the measuring cell. The coincidence circuit separates and eliminates all single photon events (i.e. noise and chemiluminescence) of the multiphoton signals generated by the radioactivity which excite both PM's. Signals which excite both PM's "simultaneously" are so-called coincident pulses which serve as useful signal for the radioactivity detector.

The radiation generated by the radioactivity is classified according to

- type
- quantity and
- energy

The ***type of radiation*** tells us whether we are dealing with alpha, beta, gamma emitters, etc.

The ***quantity of radioactivity*** is indicated in the following units:

- Becquerel (Bq = dps = decays per second).
- dpm (dpm = decays per minute, 1 Bq = 60 dpm).
- Curie (Ci = $3.7 \cdot 10^{10}$ Bq or dps).

These quantities describe how many decays per unit of time (second or minute) take place in the sample. For nuclides with shorter half-life periods (e.g.: ^{3}H, ^{125}J, ^{99}Tc), a decay correction should be carried out to determine the actual, absolute activity.

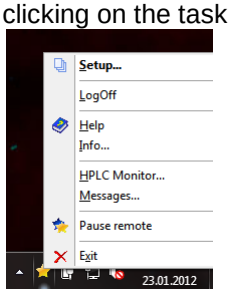
The radiation energy is dependent upon the nuclide and is given in MeV or KeV. The radiation energy of a nuclide provides information on the pulse height to be expected – depending on the scintillator used. The discriminator at the measuring system has to be set based on the expected pulse height.

12. FlowStar² Controller Software

The FlowStar² can be connected to a PC using the USB interface. This feature of the FlowStar² allows a fully remote control and parameter setup by the included HPLC monitor or additional third party software if a dedicated software driver is implemented.

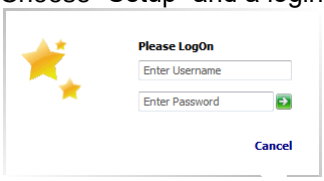
12.1 Controller

The FlowStar² controller is a standalone program which is taking care of the FlowStar² control and setup. It can be started when windows boots up and controls the FlowStar² through a free USB port. A status Icon is visible in the Windows taskbar. By right clicking on the task icon a context menu pops up:



Login

In order to get access to the user interface a login is required. Choose “Setup” and a login page will show up.



For standard users the login is “User” without password, The default administrator login is “Service” and password is “0” (zero). After the login the Controller setup page is shown.

Logoff

Log off from the backend menu. This might be required to be able to log on later with more user rights.

Help

Opens a help window with further information about the controller usage.

Info

Shows Information (type, version numbers etc.) about the instrument cell and controller software version.

HPLC Monitor

Opens a dedicated monitoring software to show the measured data as chromatogram and controls the instrument. See chapter 12.3 for more information.

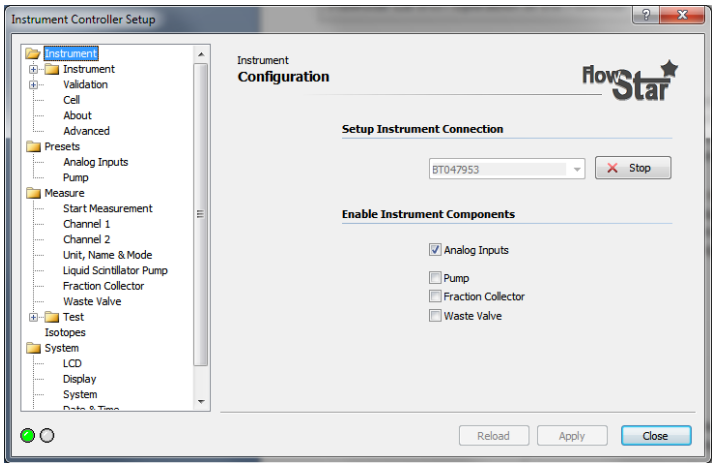
Messages
Opens a message monitor to show the error log.

Pause / Continue Remote
Allows to pause the remote control which enables the user to operate the FlowStar² by using the touch screen. While remote control is the touch screen locked. “Start Remote” will reestablish the remote control status and locks the instrument again.

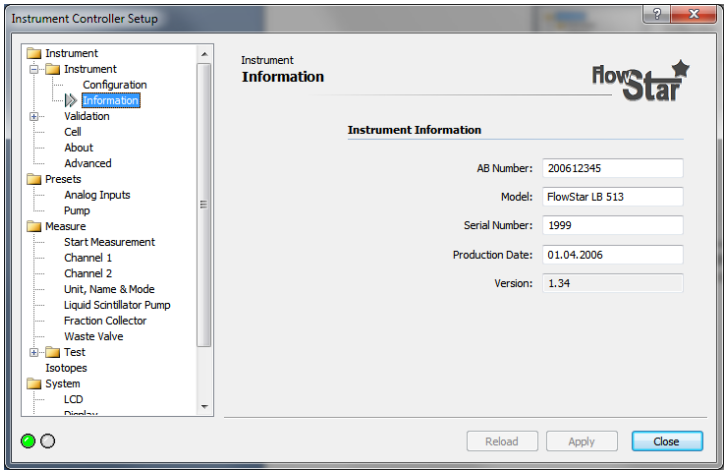
Exit
Shuts down the FlowStar² controller.

12.2 Controller Backend

Instrument

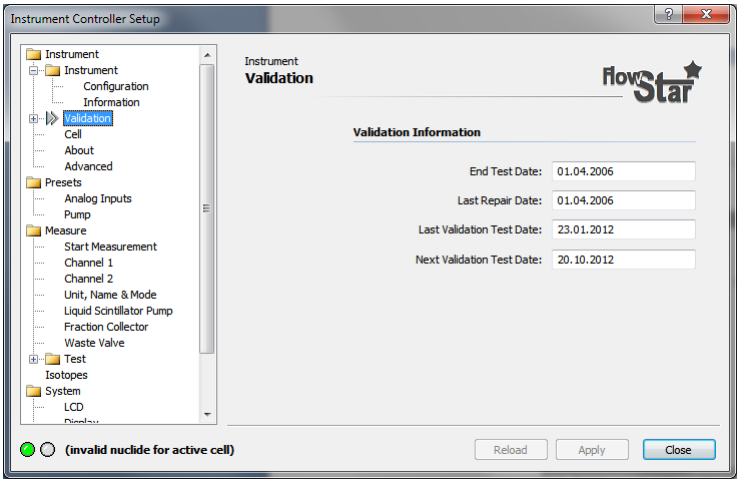


In the tab “Instrument Configuration” the current hardware connection as well as the installed features can be seen.

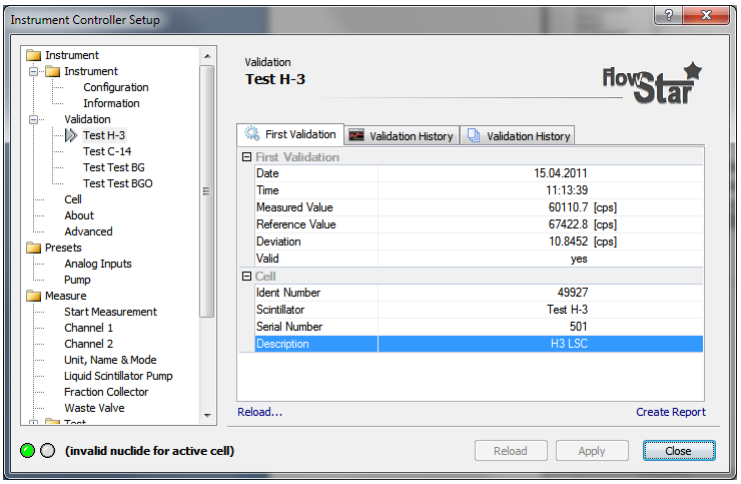


Tab “Instrument information” shows the instrument information such as the order confirmation number of the instrument, model, serial number, production date and installed firmware version number. These information are read only and cannot be overwritten by the controller.

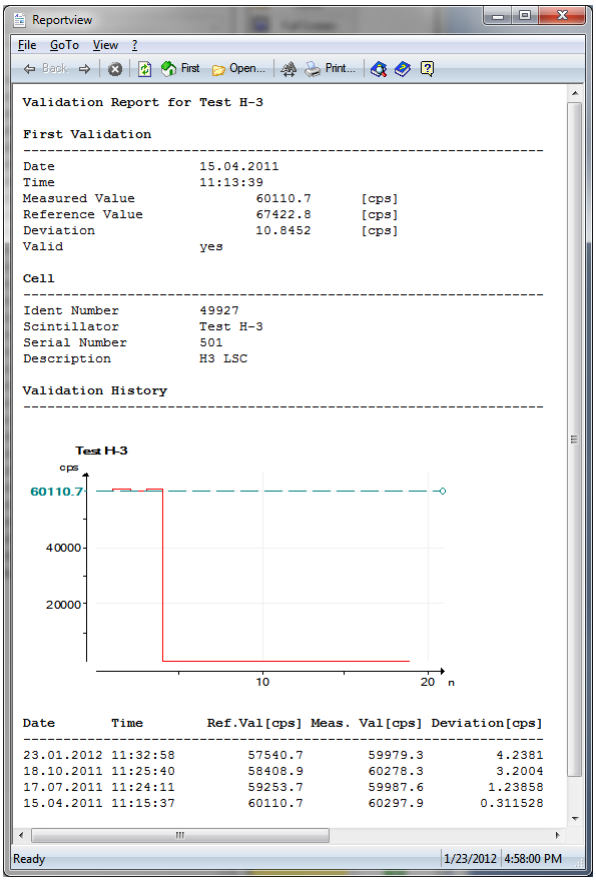
Validation



The “Validation” tab shows the dates of the validation process as well as upcoming test dates. In the subcategories Test H-3; Test C-14; Test BG; Test BGO the test results of the validation measurements as well as the first validation can be reviewed as a table or graph.
For further information regarding the validation refer to chapter 6.

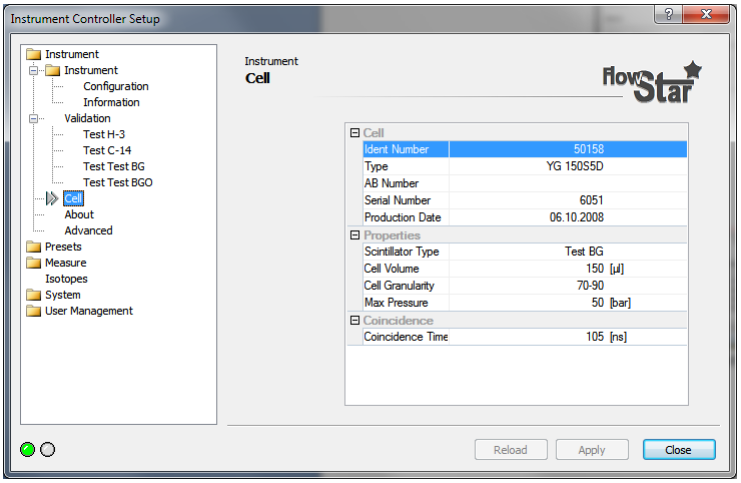


The graphs and reports can be created by clicking on the “Create Report” link below the data window. The validation report will look like this:



To print this report click on the printer icon in the icon bar.

Cell

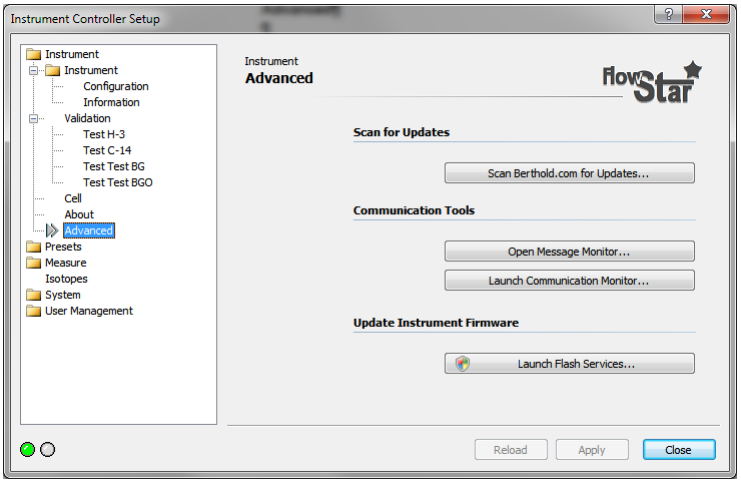


Cell information is available in the “Cell” tab. All information such as ID number, serial number, production date, cell type, volume etc. which are stored on the chip in each individual cell are displayed in this window.

About

The version number of the FlowStar² Controller and other instrument information can be seen in the “About” window

Advanced



Advanced tools for software updates and monitoring are offered in this section. By clicking 

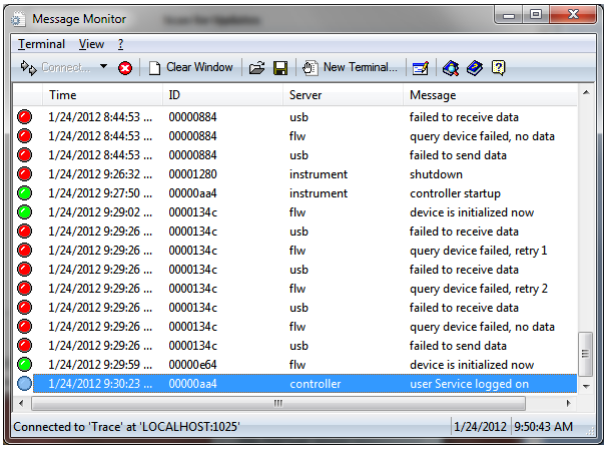
the controller connects with the Berthold Technologies webpage and checks if a newer version of the controller is available and downloads it after confirming.

The tool "flash services" is only to install a new firmware version on the FlowStar².

Please do not use this feature, unless advised by the Berthold Technologies support.

Message Monitor

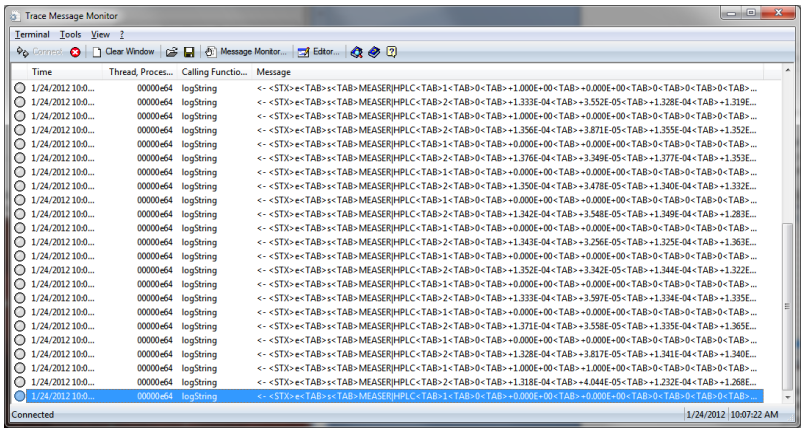
The “Message Monitor” is a log function to identify problems. After clicking on [Open Message Monitor…] opens a monitor program showing a list of log entries with login information, error codes etc.



This list can be saved as a .trs file by hitting the floppy disk icon. This file is useful for the Berthold Technologies Support to identify communication problems and should be sent by email if this is requested by the suppport.

Communication Monitor

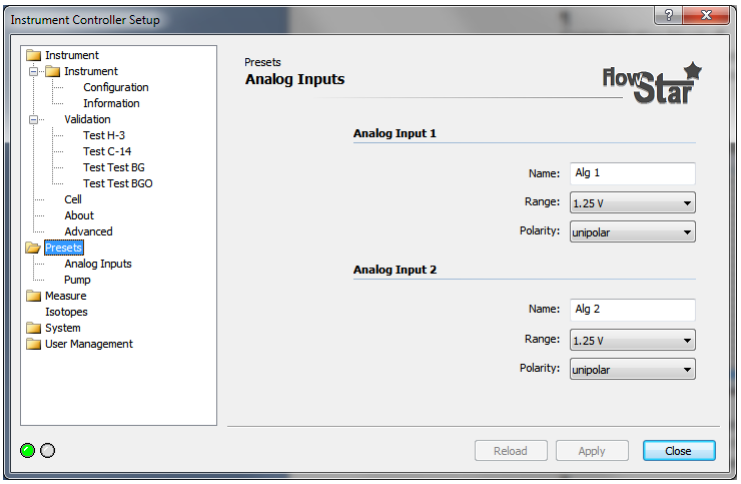
If there are general problems with the instrument communication (characters get lost etc.) the message monitor can be used to record the instrument communication. The Monitor opens when clicking on [Launch Communication Monitor…]



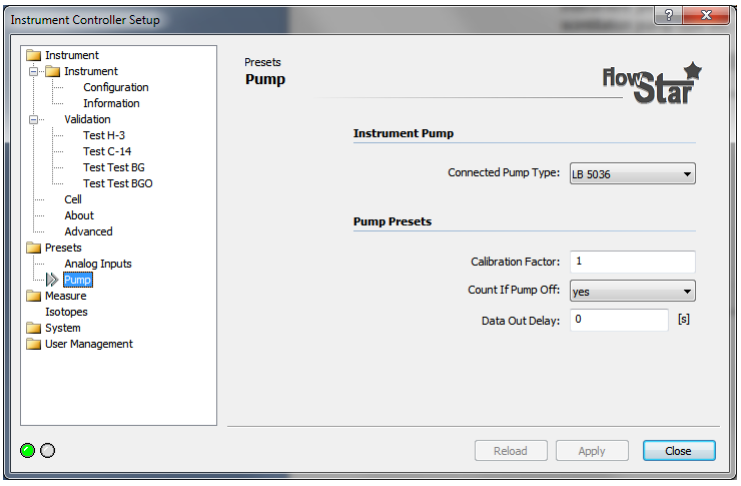
This list of commands can be saved as a .trc file by hitting the floppy disk icon. This file is useful for the Berthold Support to identify communication problems and should be sent by email if this is requested by the suppport.

Presets

In this section the Instrument presets like the setup of the analog acquisition channels, the scintillation pump type etc. are defined.

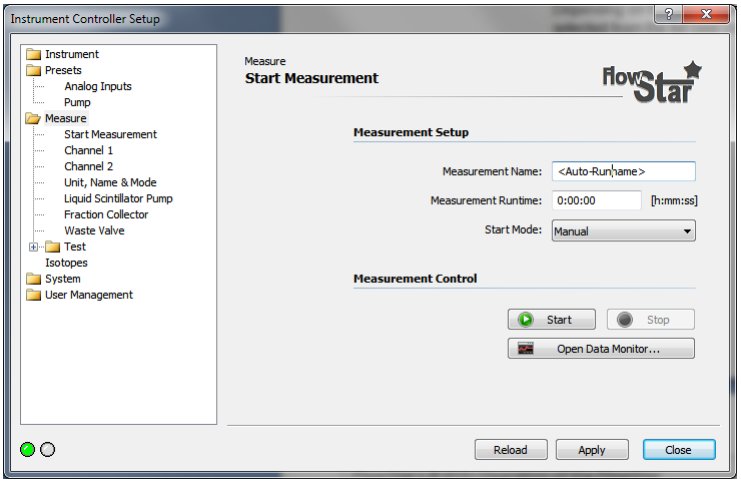


The label of the signal, the voltage range and poliarity can be defined for each individual channel (see chapter 9.1).



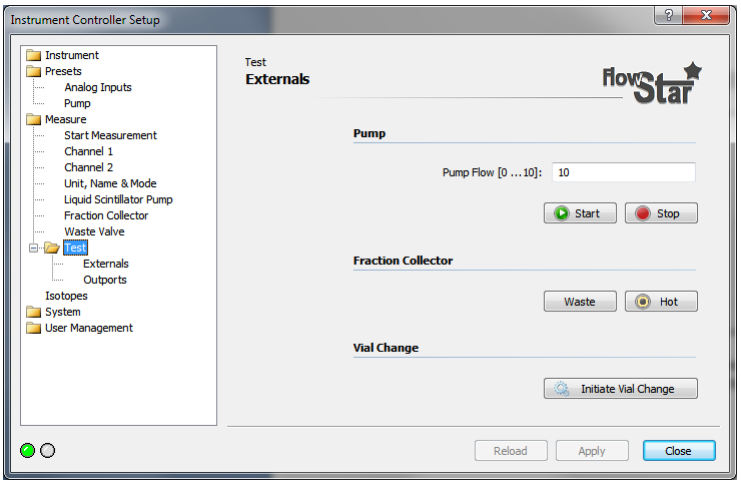
Depending on the pump is used with the FlowStar² the correct type must be selected from the list (see chapter 9.1).

Measure

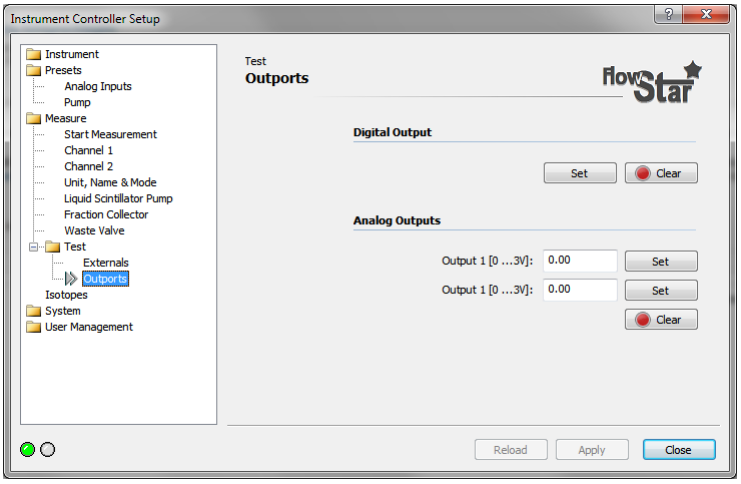


In this section the current measurement parameters can be reviewed and changed if required. For further information about the measuring parameters refer to chapter 9.4

Test



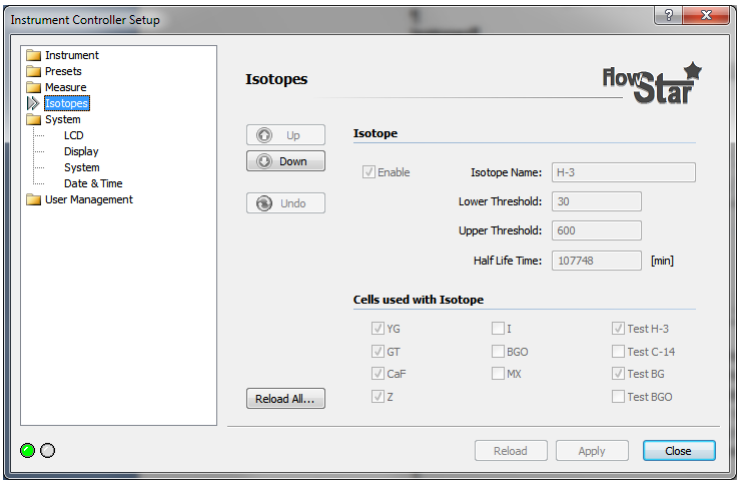
The Test features can be used during setup the instrument or to check if all accessories are properly connected and working.
The Externals section offers testing functions for the scintillation pump, the Fraction collector as well as the waste valve. A flow rate for the pump can be set and the pump can be started and stopped. According to the configuration the pump is controlled through the serial port (LB 5035-3, LB 5035-3M or LB 5037) or via the analog output and status signals at the “Scint Pump” Connector.
By clicking Waste or Hot the relay output of the waste valve / fraction collector is switching to check if the valve is working.
Clicking on Initiate Vial Change will activate the fraction collector vial output to change to the next vial.



In the Outport section the digital output (AUX) and analog outputs (Ratemeter) can be tested.
Clicking on Set will set the AUX output, Clear will reset the port.

To check the analog ports just enter an output voltage (e.g. 1V) and click on Set to have this voltage present at the output. A click on Clear will reset the output. This feature is very useful to check if the analog output port which might be connected to an external HPLC system through an A/D converter works properly (output value and signal polarity).

Isotopes

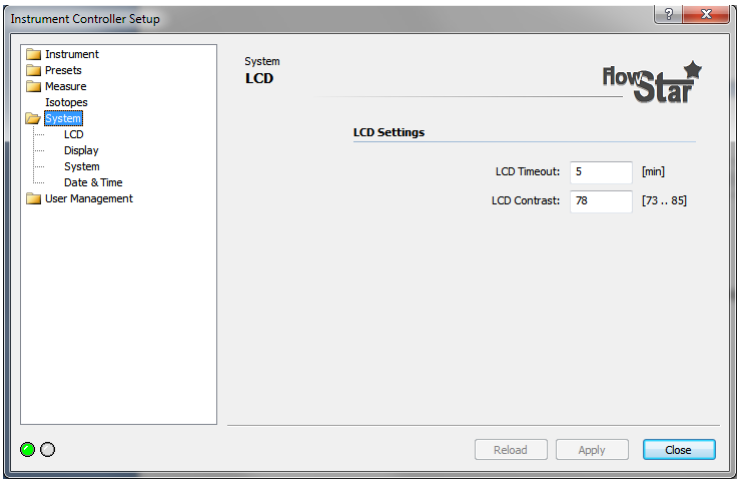


The isotope editor is a tool to browse through the table of nuclides which are stored in the FlowStar². The table of nuclides for the Standard nuclides is read only and cannot be modified. User defined isotopes can be modified. The isotope name, upper and lower threshold for the discriminator and the isotope half life time

is shown also. In the lower section are all measuring cells (checked boxes) shown who are allowed to use with this isotope.

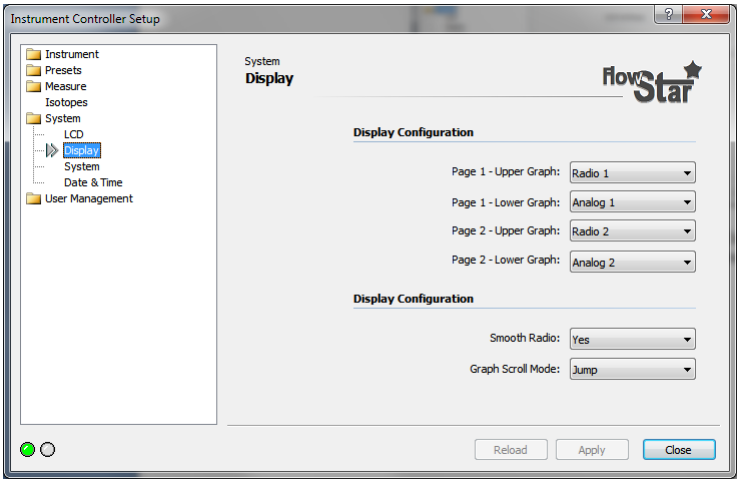
System

In the “System” tab the brightness of the LC display and the timeout for the backlight will be set.



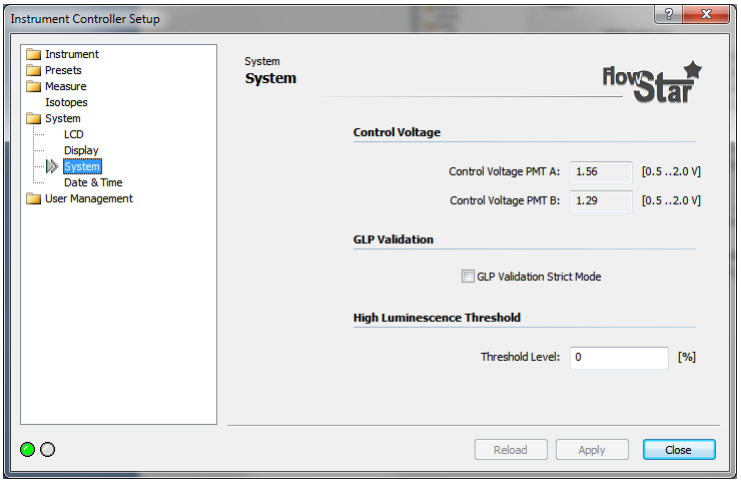
Display

To configure the FlowStar² Touch Screen use the tab “Display”. The preferences for displaying the different signals can be defined here.



To get 2 pages with full screen signal select any signal in the “upper graph” box and “none” in the “lower graph” box. For further information on these parameters please refer to chapter 9.1.

System

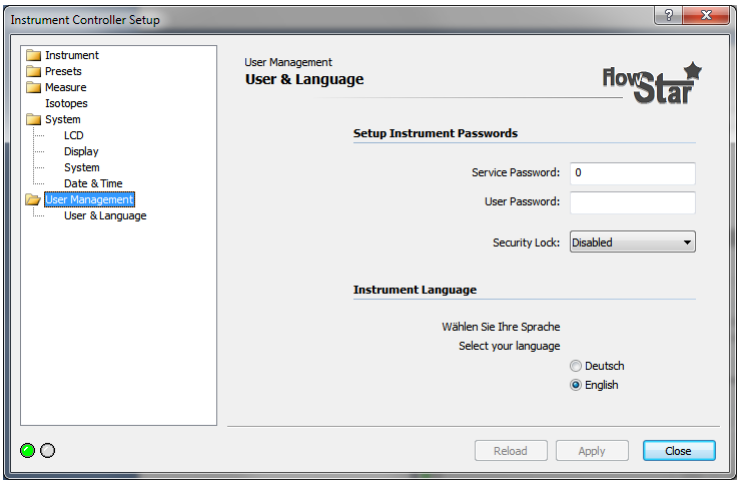


System settings like the PMT control voltage and luminescence warning levels are visible in the “System” tab. PMT control voltages are read only.
If "GLP Validation Strict Mode” is selected no further measurements will be possible if the validation is due. Luminescence warning threshold can be defined in pro cent of the signal.

Date & Time

By clicking on the Set Instrument Time to Computer Time button, the current PC time will be transferred to the FlowStar².

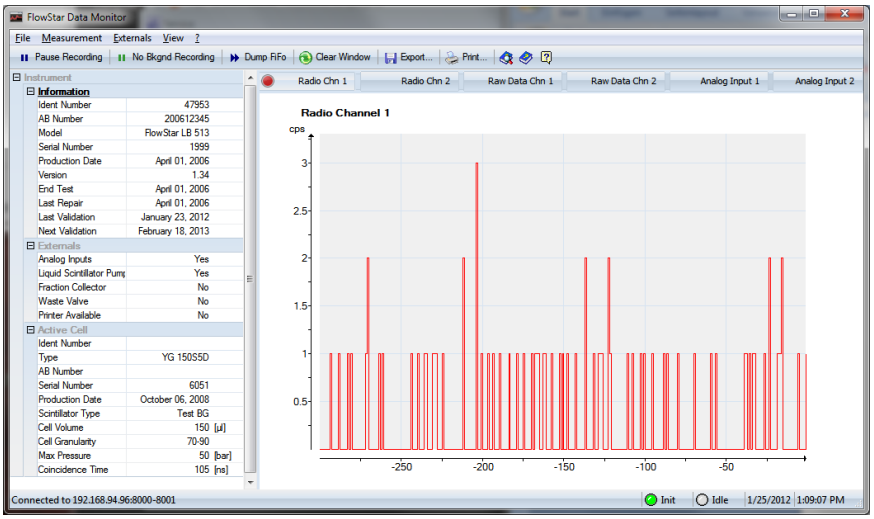
User Management



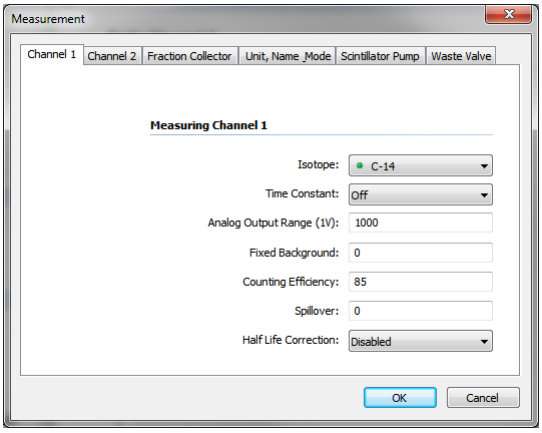
The FlowStar² has two user levels (User and Service). In this dialog box the passwords for these two users can be modified. The “User” has no deafult password, for “Service” is the default password “0” (zero).
The language for the user interface of the FlowStar² can be switched between German and English.

12.3 HPLC Data Monitor

The HPLC Monitor Software is a tool to check the instrument, run test measurements and qualify the results without using any other software.
The Data Monitor can be launched through the Windows start Menu (Programs/Berthold/FlowStar² Data Monitor) or through the context menu of the FlowStar² controller.
When launching the Monitor, the following user interface pops up:



On the left hand side is all relevant information of the instrument available while the current data are shown on the right side. The signal channels are shown in different tabs. By clicking on one of these tabs, the corresponding signal is displayed. On the left side is all relevant information over the instrument such as instrument ID, serial number, firmware version displayed.
By clicking on the Print... button the chromatogram is printed out.
In the menu “Measurement” can be selected the measurement parameters:



For more information to the parameter setting refer to chapter 12.1
In the menu “Externals” the in- and outputs (waste valve, scintillator pump, analog output) can be tested. This function is used for service purposes to check the system setup (valves, A/D converter connection etc.).

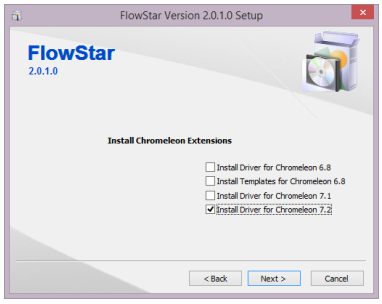
12.4 CHROMELEON® driver

The FlowStar² can be controlled by the CHROMELEON®[2] Software using an optional instrument driver provided by Berthold Technologies. This driver controls measurement parameters and acquires the measurement data.
The driver was developed and tested on Chromeleon 6.8 SP9 but works on 7.x versions as well.
To be able to use the FlowStar² driver a so called Class 3 license of Chromeleon® is required.

12.4.1 Installation

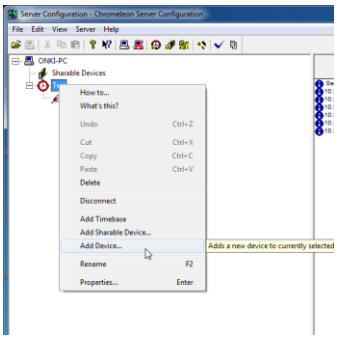
Before installing and configuring the CHROMELEON driver using the Server Configuration Tool (Instrument Configuration Manager in CM 7.x), the FlowStar² Controller must be installed. (see. Chapter 7.7). Be sure to check the relevant features for your corresponding version of CHROMELEON (see picture below).

The CHROMELEON server must be stopped during the FlowStar² controller installation.

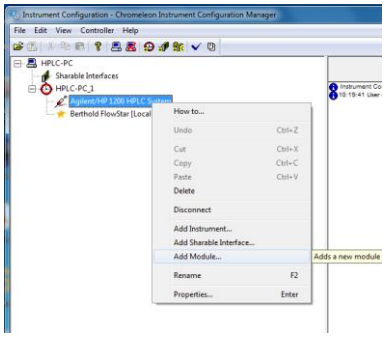


If the FlowStar² is connected to the PC and initialized properly, the instrument driver can be installed to the CHROMELEON Server using the “Server Configurator” Tool (Instrument Configuration Manager in CM 7.x). It will look like this:

[2] CHROMELEON is a registered Trademark of Dionex Corporation

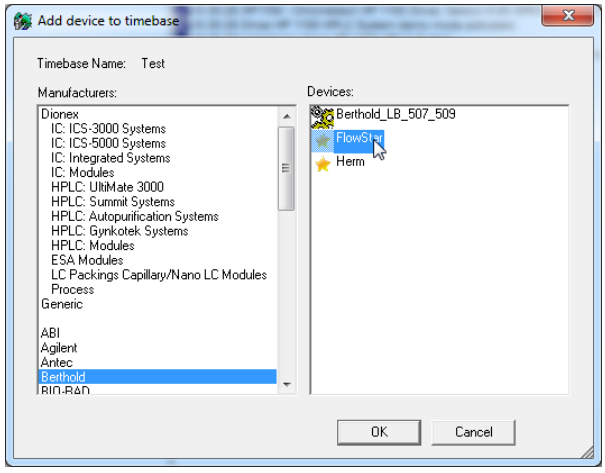


Chromeleon 6.8



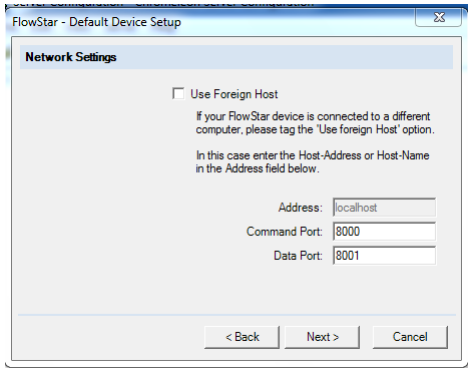
Chromeleon 7.x

By selecting the context menu function “Add Device” (“Add Module” in CHROMELEON 7.x) a list of devices is indicated. Select as manufacturer “Berthold” to get a list of supported instruments.



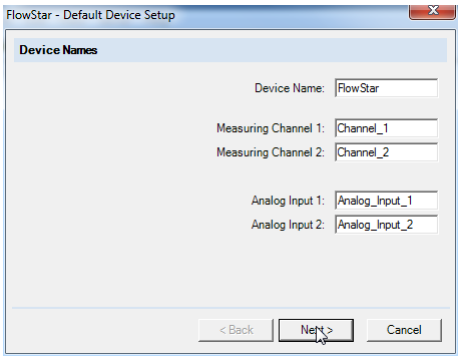
Select the item “FlowStar” from the list of devices and click OK to continue.

After a while the driver configuration dialog pops up to enter specific parameters.

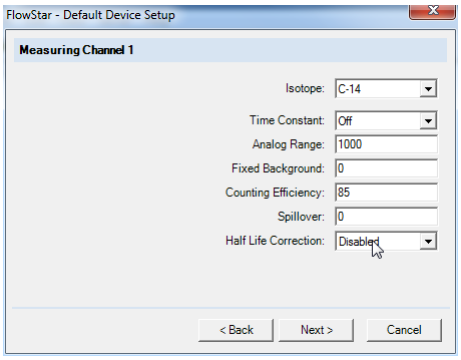


When the FlowStar² is connected to the local PC where Chromeleon is running, retain all default values for the address, command port and data port. If "Use Foreign Host” is activated the hostname of the PC must be entered on which the FlowStar² controller runs. Port numbers should not be changed. Click on

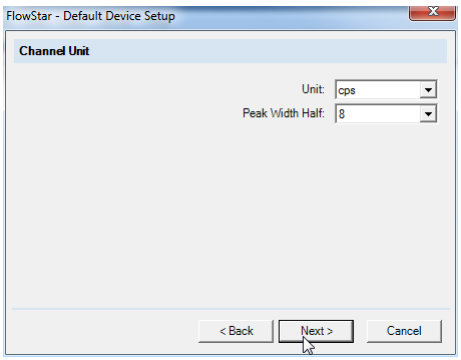
Next > to continue. The Driver will be initialized the first time. This could take a while depending on the controller status. When the initialization is finished the instrument configuration can be entered.



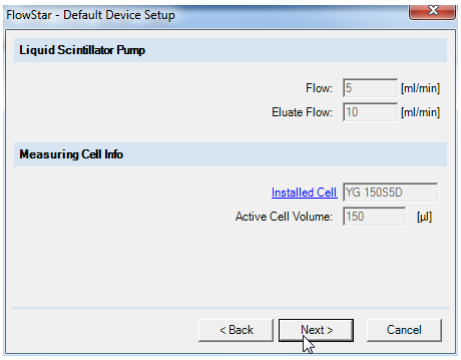
In this dialog box the Instrument and signal names can be defined. Keep in mind that a change of these names later on may require to change all your program files, because these names address the signals directly.
Click on Next > continue.



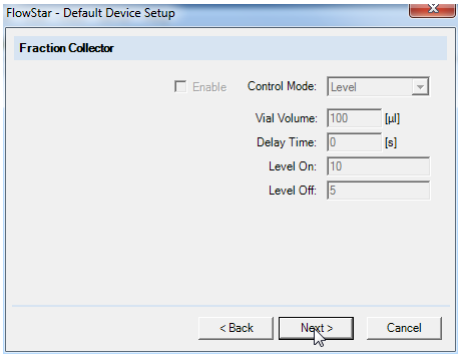
In this dialog the default measurement configuration for channel 1 can be defined. For further information about the measuring parameters refer to chapter 9.4. After clicking on Next > the parameters for channel 2 can be defined in the same way. Clicking on Next > again will lead to the measurement unit selection.



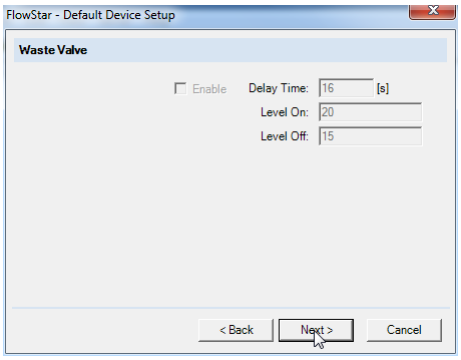
Here you can select the units (cpm, dpm, dps, Bq) for the corrected data channel. If is no correction wanted, use “cps” as unit or select the channel called “raw” channel for the acquisition. The "raw" channel shows always raw values without any further correction. By clicking on Next > the scintillator pump dialog box will show up.



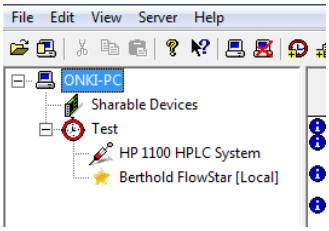
The flow rate for the pump and eluate can be defined in this box. Please note that this function is only available if a liquid scintillator pump is activated in the FlowStar² and as well a Z-cell is installed. The flow rate from the eluate is required when cpm, dpm, dps or Bq values should be calculated. By clicking on “installed cell” further information on the currently installed cell will be shown. When clicking on Next > the fraction collector parameters can be entered.



Parameter entry is only possible if the fraction collector is activated in the FlowStar² configuration. When clicking on Next > the waste valve parameters can be entered.



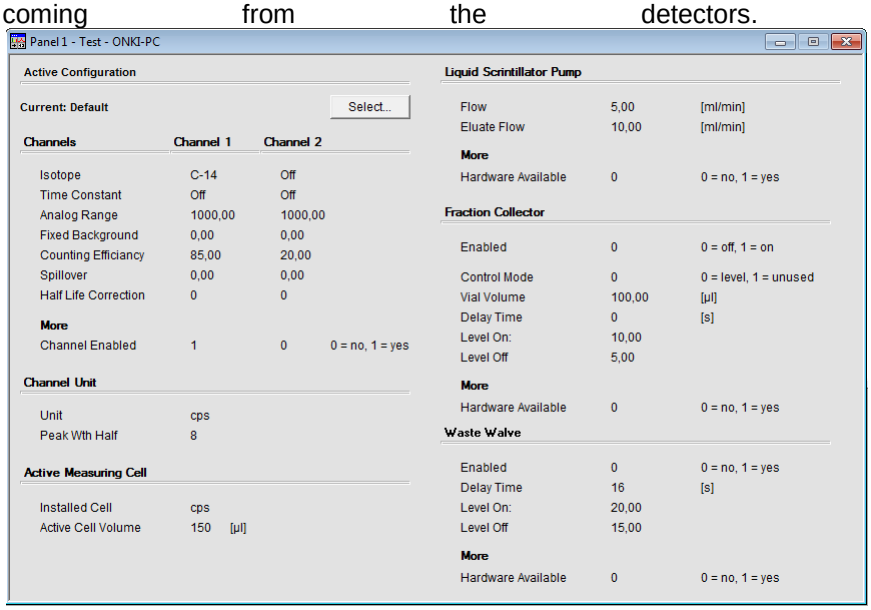
Parameter entry is only possible if the waste valve is activated in the configuration of the FlowStar². After clicking on Next > the configuration is finished. By clicking on Finish the dialog box will be closed and the FlowStar² is configured properly. The Server configuration will look like shown below, depending on the devices used:



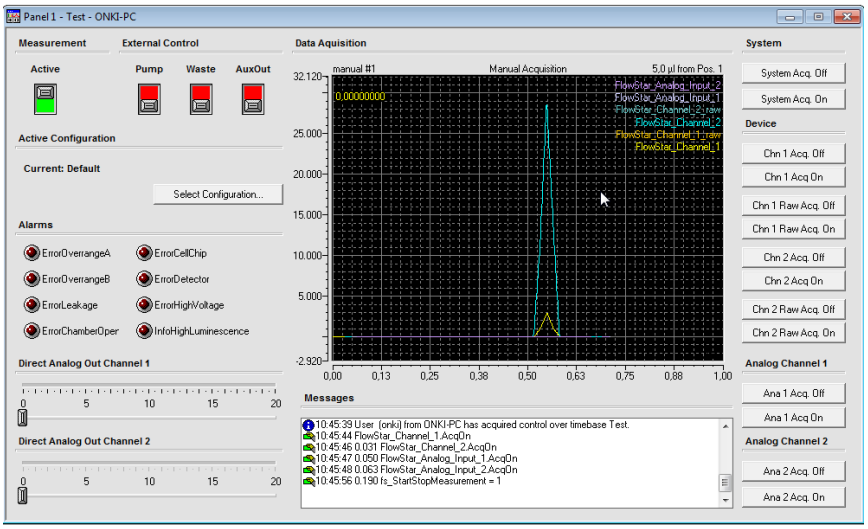
The possible different configurations (measuring cells, flow rates etc.) are saved locally at the PC where the configuration was done. This means a configuration might not be available using specific client Servers setup structures (server and server configuration done on different PCs. In this case the configuration files (*.XFS) can be copied manually from the source PC (path C:\Program Data\flwctrl\data)

When working with CHROMELEON 6.x, two different panels are available to control the instrument functions or to check data

coming from the detectors.



This panel gives an overview on the system Status and shows all setted measurement parameters. By clicking on Select... a different Setup can be selected and sent to the instrument.

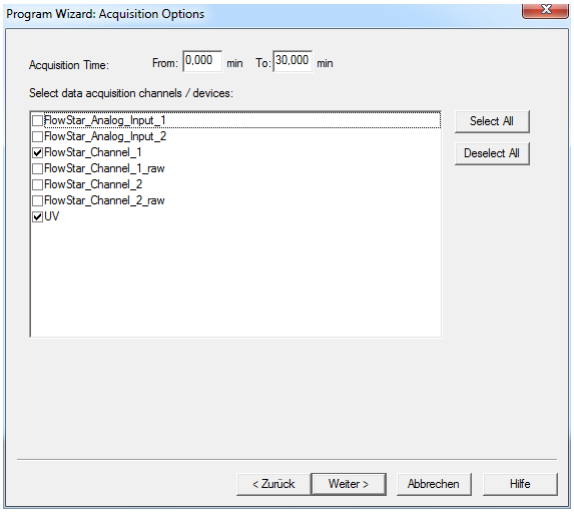


The second panel can display the count rates from the detectors and show the status of the signals. In addition several testing functions are available e.g. to set the analog output voltage to a specific value in order to test the connected A/D converter. Buttons to start / stop the data acquisition are located on the right side of the panel. If no data is shown the Acquistion state must be set to “on” and the measurement mode must be set to “active”

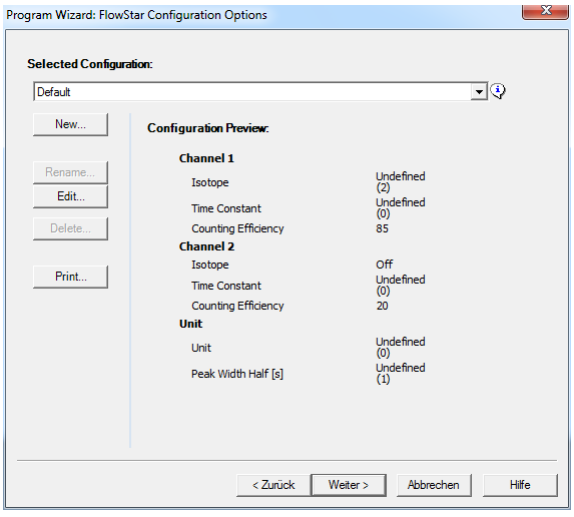
These panels are only available with CHROMELEON 6.x

12.4.2 Program File Wizard (only CHROMELEON 6.x)

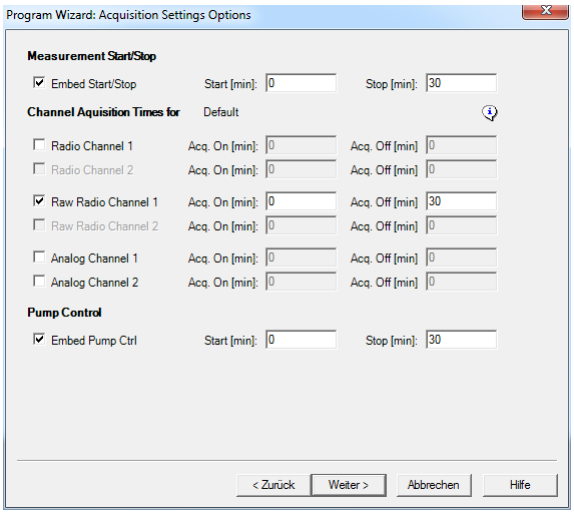
When running the program file wizard in CHOMELEON, the following FlowStar² specific dialog boxes will show up



All required signals must be checked. The channels labelled with ".raw", represents raw data channels without any calculated corrections.



In this dialog the required configuration (Isotope, efficiency, units etc.) can be selected. In case that the required configuration is not available, can be a new configuration created by clicking on New... . To modify an existing configuration click on Edit... . For print out the selected configuration for documentation purposes click on Print... .



Start/Stop times of the signal acquisition and the operation of the scintillator pump can be selected in this dialog.
When working with CHROMELEON 7.x the program file must be edited manually (see 12.4.3)

The possible different configurations (measuring cells, flow rates etc.) are saved locally at the PC where the configuration was done. This means a configuration might not be available using specific client Servers setup structures (server and server configuration done on different PCs. In this case the configuration files (*.XFS) can be copied manually from the source PC (path C:\Program Data\flwctrl\data)

12.4.3 Sample Program File

A sample program file is shown below. The FlowStar² relevant commands are highlighted.

```
fs_ActiveConfiguration =      "Default"

.
UV_VIS_1.RefBandwidth =       100 [nm]
UV_VIS_1.Step =       Auto
UV_VIS_1.Average =    On
Flow = 1.000 [ml/min]
%B =   20.0 [%]

 0.000 Autozero
Wait   UV.Ready and Pump.Ready and Sampler.Ready
Inject
UV_VIS_1.AcqOn
FlowStar²_Channel_1_raw.AcqOn
fs_StartStopMeasurement =     1
fs_PumpCtrl =  1

30.000 UV_VIS_1.AcqOff
FlowStar²_Channel_1_raw.AcqOff
fs_StartStopMeasurement =     0
fs_PumpCtrl =  0

End
```

Important Commands

- `fs_ActiveConfiguration = "Default"`
  Will use the Configuration "Default" for the run
- `FlowStar²_Channelname.AcqOn`
  Starts the acquisition of the mentioned channel. Must be placed after the "Inject" command.
- **`fs_StartStopMeasurement =  1`**
  Sends a start command to the FlowStar² to start the measurement (run time on display will start). Must be placed after the "Inject" command.
- **`fs_PumpCtrl =  1`**
  Starts the scintillator pump connected to the FlowStar² using the ramp and the flow rate set in the configuration
- **`FlowStar²_Channelname.AcqOff`**
  Stops the acquisition of the mentioned channel
  `fs_StartStopMeasurement =  0`
  Sends a stop command to the FlowStar² to stop the measurement (run time on display will stop)
- **`fs_PumpCtrl =  0`**
  Stops the scintillator pump connected to the FlowStar²

13. Explanations

13.1 Peak Half-Width - Time Constant - Smoothing

An absolutely essential requirement for a good on-line peak detection in the HPLC integrator is the best possible fit of the ratemeter signal (analog output signal) to the expected peak shape - especially the peak half-width. A good fit of the time constant is the more important the closer the activities that are to be detected come to the background level. With activities which are significantly above the background a too small time constant is usually irrelevant, while too great a time constant is always harmful for an optimum reproduction of the peaks.

A Gaussian smooth is applied to the peaks, combining a good peak detection and minor peak deformation (flattening and broadening) when working with the optimum time constant.
The smoothing method is used for these reasons:
distinction between background variations and peaks
separate presentation of overlapping peaks
smooth of statistical variations in the peak curve.

The parameters **peak half-width** and **time constant** (see chapter 9.3) ensure the optimum fit of the smooth to the peaks.
The peak half-width is used to fit the curve smooth to the expected peak width. When using a too high peak half-width, the smoothing procedure will result in a flattening of the peaks, smaller or overlapping peaks will not be detected. If this parameter is too small, random variations will falsely be identified as peaks.
In praxis the expected peak width is known. It is caused by the examined substances or the separation at the column, the cell volume, the scintillator used and the flow rate (admixture method).
The time constant specifies how many basis time intervals are used for averaging and which weight is assigned to the individual intervals.

Let us now turn to the technical realization in the FlowStar^2 LB 514:
After one second, the monitor stores the number of counts registered in the last second interval and keeps them stored for 128 seconds, so that the counts registered over the last 128 seconds can be called up any time. From the value entered for the peak half-width one derives the so-called basis time interval. The basis time interval is always one quarter of the peak half-width (the exact value is listed in Table 1).
First, the counts from the single intervals are summarized in groups (= basis time interval). The number of single intervals per

group is defined by the length of the basis time interval. A maximum of 8 groups will be formed (see table 2, column 2), always starting with the counts measured last. Since the basis time interval is max. 16 seconds long (see table 1), all counts of the last 128 seconds (128 = 8 x 16) will be summarized in groups in this case.

Peak half- width	Basis time interval
4	1
8	2
15	4
30	8
60	16

Table 1

One should also keep in mind that the number of counts in the groups may change every second, since a new second interval will be added and the one stored 128 seconds ago will be rejected. The counts change in one-second intervals even if the basis time intervals are longer than 1 second. Averaging over several second intervals results in a significant smooth of the variations.

The further procedure for calculating a smoothed average value always proceeds from the counts in these groups.
By entering one time constant one defines how many basis time intervals with their counts are to be used for averaging. The time constants 0.5, 1.0, 1.5 and 2.0 are available. Table 2 shows that 2, 4, 6 or 8 basis time intervals will then be used. Since one basis time interval is about equal to one quarter of the peak half-width, this means that the average is calculated over half, one, one and a half or two peak half-widths (the time constant is indicated in peak half-widths).

To ensure that a peak will not flatten out too much when averaging over a wide range, the basis time intervals are weighted less strongly at the beginning and end than those in the middle of the interval (see Table 2, column 3). Nevertheless, a greater time constant means that greater parts of the peak will be detected and the probability that weak peaks may still be detected will be improved.

Time constant	No. of basis time intervals	Weight of basis time intervals during averaging
0.5	2	$\frac{1}{2}$ $\frac{1}{2}$
1.0	4	$\frac{1}{8}$ $\frac{3}{8}$ $\frac{3}{8}$ $\frac{1}{8}$
1.5	6	$\frac{1}{32}$ $\frac{5}{32}$ $\frac{10}{32}$ $\frac{10}{32}$ $\frac{5}{32}$ $\frac{1}{32}$
2.0	8	$\frac{1}{128}$ $\frac{7}{128}$ $\frac{21}{128}$ $\frac{35}{128}$ $\frac{35}{128}$ $\frac{21}{128}$ $\frac{7}{128}$ $\frac{1}{128}$

Table 2

13.2 Efficiency Correction

The efficiency correction is affected by the parameters 5. through 12.
By taking into account the empirically determined static and dynamic efficiency, the cpm or cps values calculated as a function of the ratemeter unit can be converted such that the activity flow is output in dpm/min or dps/min; with a time axis in minutes the integration of the peak area results directly in the activity contained in the peak. In the following we will assume that the ratemeter unit has been set to minutes, so that we will get cpm/min or dpm/min. With the ratemeter unit seconds, cpm has to be substituted by cps and dpm/min by dps/min.
Essential requirements for the activity calculation is the correct calculation of the net cpm values, i.e. the values for the background rates and the spillover factor must be determined correctly and entered accordingly for the inquiries 5. - 7. The net cpm values are calculated as follows.

cpm_{net} = cpm_{gr} - Background [cpm]

dual Ch cpm_{net} = cpm_{gr} - Background [cpm] - H-cpm_{net} Spill Ch1 → Ch2 [%]/100 [%]

These formulae show that for dual channel measurements the net cpm value of the higher-energy channel must be calculated first, since this value is required for the spillover correction.
For single channel measurements the formula is identical to the first formular mentioned above:

cpm_{net} = cpm_{gr} - Background [cpm]

If one gets negative values for the net cpm, they will be set to zero for output or further processing.
The calculated net cpm values are now used for calculation of the activity flow according to the following formula:

$$dpm/\min = \frac{(F_{El} + F_{Sc})\,[\mathrm{ml/min}]\cdot 100\,[\%]\cdot 1000\,[\mu l/\mathrm{ml}]}{\mathrm{Eff}\,[\%]\cdot \mathrm{Vol}\,[\mu l]}$$

where:
F_{El}: eluate flow coming from the HPLC system
F_{Sc}: scintillator flow (only relevant for admixture cells)
Eff: static efficiency of the installed measureing cell
Vol: volume of measuring cell taken from the cell ID chip.

Through integration of the activity flow over the entire peak one gets the activity of the individual peaks. With a time axis in minutes, the activity in dpm corresponds to the area of the peak.

14. Disturbances

This chapter summarizes all possible disturbances, describes them, explains their cause and provides information on how to eliminate them.

14.1 Electrical or Mains Disturbance

Sparks generated by old refrigerators, centrifuges, electrical switches may enter the measuring instrument through the mains supply or cables and cause disturbances in the detector or in the digital unit.

The following steps should solve the problem:

- Connect all units which are part of one measuring system (HPLC pumps, gradient controller, UV detector, integrator, radioactivity flow-through monitor, computer, etc.) to one mains phase.
- Check mass connection, eliminate any possible crossover resistance or loose contact.
- We recommend using mains suppression filters (Schaffner filter - supplied by our Service Department).

14.2 Background (BG)

Table 2 lists typical background values. These values may differ slightly depending on the setup location.

Cell	cpm
YG-150	18 - 20
YG-400	20 - 25
Z-200	5 - 8
Z-500	5 - 8
Z-1000	6 - 9
J-1000	60 - 100
BGO-X	80 - 120

Table 2: Typical background values for individual measuring cells
The values are valid for brand-new (unused) cells. The typical BG value is affected by various factors (see Figure 35).

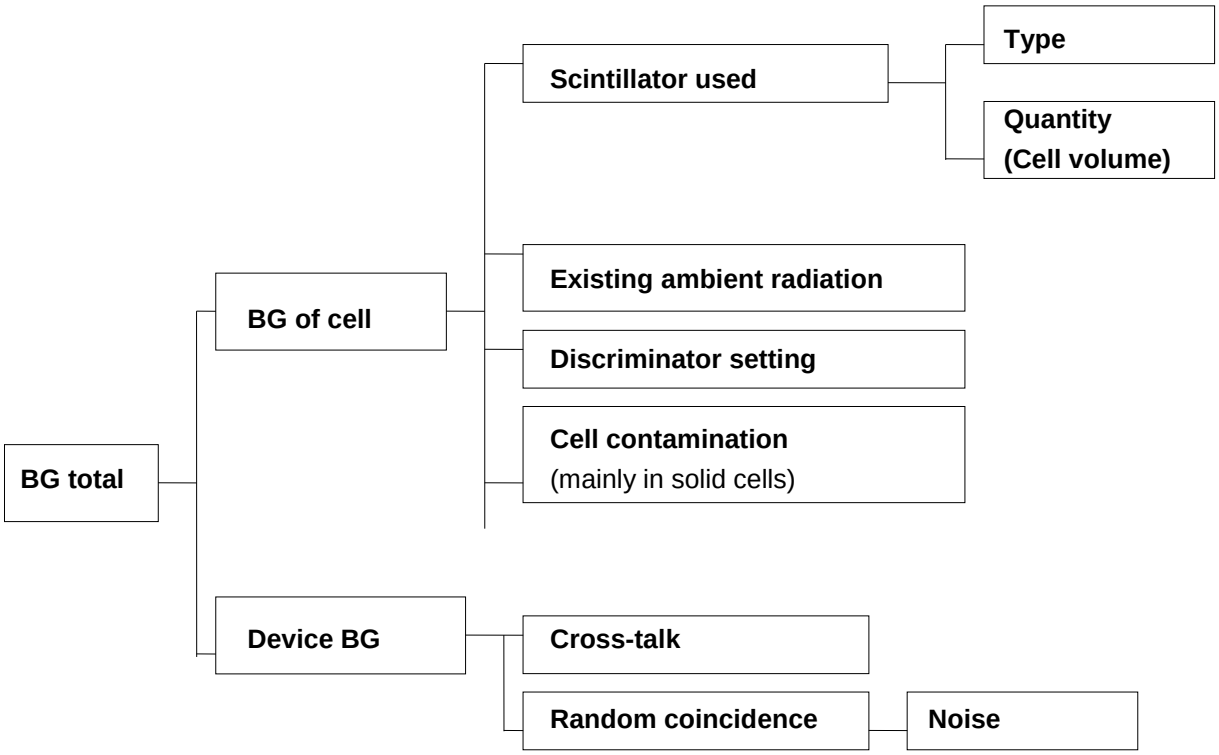


Figure 35: Factors affecting the background

If the BG values are significantly higher than the values listed in the table, check first whether this is caused by the measuring cell or the instrument:

- If the measuring cell is the cause, replace it by a well-flushed or new empty admixture cell (Z-1000 or Z-500), where the in- and outlets are well-protected from incident light by aluminum foil (even better: use steel capillaries). In this case, only the “instrument BG” has to be measured. For service personnel we recommend measuring the individual rate of PM A(left) and PM B(right) in the “SERVICE” menu. The individual rate is normally between 50 and 450 cps. If the measurement chamber has been open for a long time, these values may be higher. **Caution:** Both PM’s always have different single rates!
- Up to 1000 cps will be compensated without any problem by the coincidence circuit. Higher single rates in both counting channels are caused by insufficient light protection or errors in the instrument.
- If the single rate is higher in one counting channel only, this may be caused by the high voltage or the PM.
- The “instrument BG” is OK if the values specified are measured with the empty, clean admixture cell (Z-500 or Z-1000), even though the BG increases after installation of the new measuring cell.

Possible reasons and solutions:

- The newly installed solid cell has not yet decayed (mainly with YG, J-1000, BGO-x cells).
  Wait until the cell has decayed.
- The solid cell is contaminated.
  Replace cell or decontaminate it.
- Light incidence in counting chamber.
  - *O-ring seal defective (replace it on the front side of the counting chamber)*
  - *Teflon or other plastic capillaries (e.g. PEEK capillary) are used as inlets or outlets. Cover capillaries with black shrinking tube or insulation tape (several layers, min. 30 cm) or use stainless steel capillaries.*
    Please note that PEEK capillaries are not light-tight! A black 15 cm long black protective hose may help. We recommend using “Norprene®”, a black silicon like hose with an external diameter of 4 mm and an internal diameter of 2 mm to cover the PEEK capillary.
    The Norprene® hose is used with the scintillator pump (LB 5037) as inlet hose.
  - *Luminescence effects in cell (see next section)*
- Increased BG after start of eluate.
  Activity is continuously washed off the column (column is bleeding).
  Check: collect 1 ml eluate and measure it in LSC1.

14.3 Luminescence Effects

This chapter summarizes all those effects which cause a signal in the radioactivity channels even though no radioactivity is present in the measuring cell. The spectral distribution (channel ratio) indicates the cause. Depending on the spectral distribution, the following phenomena may occur:

- Message: "**High Luminescence**" in the status window.
- apparent or ghost peaks
- unquiet and increased BG line, with luminescence warning
- unquiet and increased BG line, with additional outliers, spikes
- rising rate with some purely organic solvents; this effect usually occurs with a rising flow rate and decreases when turning off the flow rate (decays very slowly; decay time may be up to one hour).

Luminescence refers to light events occurring so often that they cannot be compensated any more by the coincidence circuit and, consequently, may disturb the measurement process in the measuring cell. These single photon events occur mainly in the low-energy channel but cause no interference in the high-energy channel with increased lower level LL = 050 to 100:

Phosphorescence

Some organic solvents are excited by external light and emit the accumulated light in the cell.

Remedy:
Keep solvent in a dark container, cover transparent supply lines (e.g. Teflon) by black foil.

ACN Step Gradient

With acetonitril-buffer-gradients in the range 60 - 80% acetonitril concentration. A ghost peak may occur if a step gradient is used in this range. The effect occurs with solid cells as well as with the admixture method. The cause is that the acetonitril is not completely mixed with the buffer behind the column. Streaks are visible.

Remedy:
Select a less steep gradient curve.

Luminescence caused by incomplete mixing

The mixing process in the measuring cell is not yet completed. One reason is, for example, that the scintillator mixes only badly with the solvent. In addition to heat, light and luminescence are also generated. Open the measuring cell inlet, put the stainless steel capillary in a transparent glass container and let the cocktail flow off inside the glass. An incomplete mixing process is indicated by the fact that streaks are still noticeable.

Remedy:
In most cases the desired homogeneous mixture can be obtained with a longer (30 - 50 cm) pinch-type capillary mixer.
Increase, in some cases “reduce”, the scintillator share in the cocktail.

Flash effect

Interference in solid cells. With some purely organic, non-conductive solvents (n-Hexan, chloroform) static charge effects are noticeable at a higher flow rate. The friction of the molecules produces a static voltage which is not carried off due to the high resistance of the eluate. Consequently, the voltage is discharged and high-energy flashes of light (flash effect) occur which cause interference in both discriminator channels. The interference still persists even when the flow rate has been stopped and decreases only very slowly.

Remedy:
1. Interference disappears suddenly when adding a small amount (1-3%) of conductive solvent (e.g. Methanol).
2. Use “D”-cells which include a “conductor” on the inside, e.g. the cells YG-150 D, YG-150 U5 D.

15. Troubleshooting Check List

General Information

Error description	*Possibly cause/Remedy*
No function, Display or LED dark	Check mains voltage, mains connection, power on switch, mains cable, fuses.
Measurement OK. Display "0 cpm"	Compare PM HV control voltage value with datasheet (delivered with the instrument). PM function check: measure single photon rate and efficiency.

Display "Error" when the <Status> button is pushed:

The measurement is stopped automatically if "**Error**" is displayed.
Push the <**Status**> button to view further information on the error:

Error info after pushing the ***<Status>*** *button*	*Possibly cause/Remedy*
Leakage	Moisture sensor has detected liquid inside the device. Turn device off immediately and separate it from mains. For information on how to proceed please refer to Chapter 3.5 Cleaning the Counting Chamber.
Counting chamber open	The counting chamber is not completely closed. Check if the measuring cell has been inserted correctly and fixed with screws.
Detector A+B HV off	Detector does not get any or too little high voltage. Check the HV parameters.
Detector error	Check the detector and HV parameters. If the error persists, the detector may be damaged. Please contact Berthold's local service office.
Cell chip error	a) Device cannot read the cell chip properly. Check if the chip contacts are clean. Test another measuring cell. If the error cannot be remedied, please contact Berthold's local service office. b) The inserted measuring cell does not fit the configuration. Please change the configuration accordingly or insert another type of measuring cell.

Display "Warning" when the Status button is pushed:
The measurement is not stopped if "**Error**" is displayed.
Push the **Status** button to view further information on the error:

*Error info after pushing the **Status** button*	*Possibly cause/Remedy*
High luminescence	Caution! High luminescence! (see chapter 14.3)
Overflow detector A/B	Luminescence too high. The result of the measurement cannot be processed any more.

Display "Error" when the <Cell> button is pushed:
The measurement is stopped automatically if "**Error**" is displayed.
Push the <**Cell**> button to view further information on the error.

Display "Error" when the < LS-Pump> button is pushed:
The measurement is stopped automatically if "**Error**" is displayed.
Push the **<Pump>** button to view further information on the error.

15.1 Instrument cleaning

The instrument can be cleaned using a soft cloth to remove dust etc.. In case of significant staining a light detergent can be used with water.
Make sure no liqid gets inside the housing during cleaning.

Pull the mains plug before cleaning.
Make sure the instrument and its surfaces are completely dry before re-connecting the mains plug.
Don’t use abrasive powder for cleaning as this could cause scratches on the surfaces.

16. Technical Data

Detection Unit
State of the Art ultra sensitive 2 inch PMT detection system with random coincidence counting and luminescence subtraction.

Display
Monochrome graphical touch screen with 320 x 240 pixel resolution

Communication
USB 2.0 (B-Type connector), RS-232 port for scintillator pump control

Inputs
Start- stop- and auxiliary-signal (TTL).
2 analogue inputs (24 bit resolution) with variable voltage (bipolar).

Outputs
Ready- error and luminescence warning output signal (TTL open collector).
Scintillator pump control with status signal and voltage controlled flow rate.
2 analogue signal outputs 0-1V (2.5 times oversampling).
Waste valve and fraction collector control output.
12V Supply voltage (max rating. 100mA per port)

Software
Built-in software operated with touch screen or external control and evaluation via RadioStar or Chromeleon driver.

Power Supply
90 – 264 VAC, 50/60Hz
Power consumption during operation approx. 20 Watt

Temp range
Storage: 5-40 °C
Operation: 15-35 °C

Humidity
10-90% non condensing

Dimensions
410 x 170 x 410 mm (WxHxD)
A minimum of 100mm distance to any wall or instrument at the rear panel as well as a flat bottom place is required for optimal ventilation of the instrument.

Weight
Approx.. 16kg

17. Index

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