Sirius L Single Tube Luminometer

User's Manual

Sirius L-e-2011-11 © Berthold Detection Systems GmbH, 2011



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1. Preface

1.1 Contact information

Berthold Detection Systems GmbH Bleichstraße 56-68 D-75173 Pforzheim

Sales and Customer Service

Internet:	www.titertek-berthold.com
Phone:	+49 (0) 7231-9206-0
Fax:	+49 (0) 7231-9206-50
E-Mail:	sales@titertek-berthold.com

Instruments Technical Support

Phone:	+49 (0) 7231-9206-25
Fax:	+49 (0) 7231-9206-50
E-Mail:	service@titertek-berthold.com

1.2 Typographical Conventions

This manual uses the following typographical conventions:

lcon	Description		
	This caution sign calls attention to important safety information to be read in the manual.		
	The caution sign indicates the potential for risk of electric shock.		
ī	This symbol advises the reader to consult the operating instructions for information needed for the proper use of the device.		



Icon	Description
	Manufacturer
CE	This product bears the CE mark.
ī	This symbol advises the reader to consult the operating instructions for information needed for the proper use of the device.
	Dispose the instrument according to Directive 2002/96/EC, on waste electrical and electronic equipment (WEEE)".
	This caution sign calls attention to important safety information to be read in the manual.

These icons may appear on the instrument:

1.3 Revision History

Date	Changes
2010-03	Initial document
2011-11	New manual template. Revision of Logo, contact information and typographical conventions. Transport conditions and disposal of biohazard waste added.

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1.4



Intended Use Statement

Description of Sirius L

The Sirius L tube luminometer is a highly sensitive and compact single sample luminometer, designed specifically for the detection of chemiluminescence and bioluminescence in different kinds of applications. Due to its small footprint it can be set up in any lab workplace. In its basic version it can be employed for all glow type measurements. Equipped with injectors, the instrument may also be used for flash luminescence measurements. The sample chamber accommodates various sample formats. Sirius L is operated using special Windows software.

Intended Use:

The Sirius L Tube Luminometer combined with FB12/ Sirius software may be used for the following purposes:

Academic research, e.g. biology, chemistry, human and veterinary medicine.

Hygiene control, e.g. water measurement.

Pharmaceutical industry, e.g. drug discovery research, quality control in production.

Sirius L may only be used for measurement of bio-and chemiluminescence in single samples. No others than the purposes described in the Intended Use may be operated on the system.



Constraints:

Sirius L with FB12/ Sirius software must not be used for In vitro diagnostics.

1.5 Certi CE This is current

Certifications

This instrument bears the CE mark, based on conformity to current EC legislation.



1.6



Safety Instructions and Precautions

Please adhere to the following safety instructions and precautions before and during operation of the system or taking the instrument into service:

- 1. The instruments have been tested by the manufacturer and are supplied in a condition that allows safe and reliable operation. The manufacturer guarantees safe operation of the equipment, both electrically and mechanically, if user follows the instructions set forth in this manual.
- 2. The person, responsible for bringing the instrument to market, has to assure, that the safety instructions and precautions of this manual are communicated to the user.
- 3. Only qualified trained personnel may operate the instrument.
- 4. It is strongly recommended that all users read this manual prior to use. This User's Manual includes information and warnings that have to be observed by the user to ensure safe operation of the instruments.
- 5. It is the operator's responsibility to adhere to regulations on the installation and/or operation of sample measuring systems that are required by local legislation of the country of its installation.
- 6. The user must ensure that the instruments are set up and installed in such a way that their function is not impaired. Please refer to the luminometer installation description.
- 7. Only accessories coming with the instrument or delivered by Berthold Detection Systems for work with this instrument may be used for operation.
- 8. Do not connect the power cord near fluids to avoid electric shock and burning. The power cord must never become wet!
- 9. The power supply must be connected to a wall outlet complying with local regulations of the country of its installation and providing voltage and current according to the specification of the system.



- 10. **Berthold Detection Systems** assumes no liability for any damages, including those to third parties, caused by improper installation, use or handling of the device. The instruments are live and improper handling may cause damage.
- 11. The instrument may only be used for the designated application. Please refer to the Intended Use Statement and the Constraints.
- 12. The user must assure that assays are validated with the system prior to routine use.
- 13. Some assays, assay components or specimen may pose a biohazard or risk for infection. Always refer to the assay's package insert for adequate safety precautions. Wear appropriate protective equipment like laboratory coats or chemically resistant rubber cloves and act carefully to avoid contamination.
- 14. The instrument must be decontaminated before repair work or service to avoid contact of the service personnel with potential biohazard material.
- 15. Only service engineers authorized by **Berthold Detection Systems** may carry out service and repair work. Before continued use reassemble and check the instrument according to instructions in the service manual.
- 16. For servicing use only parts qualified by Berthold Detection Systems.
- 17. Always disconnect the power cord before opening the instrument for service or modifications.
- 18. The user may only perform the maintenance work described in this manual. The user must not open the instruments chassis.
- 19. The tests and maintenance work recommended by the manufacturer should be performed to make sure that the operator remains safe and that the instrument continues to function correctly.
- 20. If you realize that the instrument has become unsafe to use, switch it off and disconnect it from the power supply.



- 21. Avoid spilling liquids on the outer surface and the sample chamber of the instrument. Wipe up all spills immediately and decontaminate the surfaces in cases of biohazard spilling liquids.
- 22. If liquid gets inside the instrument, pull the power cord immediately. Do not operate the instrument if internal components have been exposed to fluids, since they create a potential for electric shock and burning. Have the instrument cleaned by an authorized service center.

Risk Management Statement

Sirius L with and without injectors is subject to a continuous risk analysis and evaluation according to EN 14971. The information in this manual complies with the actual state of knowledge at the publication date. When Sirius L is operated in compliance with the instructions in this manual there are no known risks for the user, the environment or the quality of the measurement results. However, the user should be aware of situations that could result in serious damage. Always read the safety instructions and precautions carefully!



Storage conditions

Before delivery or if the instrument is not used for a longer period of time, store it in the original cardboard box in a dry dust-free environment and protected from direct sunlight and significant temperature fluctuations!

Storage temperature: 0-40°C up to 80% humidity (@30°C), non condensing



Transport conditions

-25° to +60°C, up to 75% humidity, in original cardboard box and free of liquids.

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Quality control

It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the package insert of the reagent kit or the standard laboratory protocol for the test to be conducted. A failure in the performance of Quality Control checks could result in erroneous test data.

Samples should be obtained, treated and stored following the instructions and recommendations of the kit insert.

It is recommended to run known internal quality standards or samples attendant to the measurement runs or to use the LED TestTube, offered by Berthold Detections Systems, for instrument validation measurements.



Return shipment

If the instrument has to be returned to Berthold Detection Systems for servicing or inspection, we recommend that you use the original cardboard box. Refer to the chapter 14 for details. Always decontaminate the instrument according to the description in the decontamination form in chapter 15. Fill out this decontamination form before return shipment. Berthold Detection System will not accept instruments without filled out decontamination form for repair or inspection.



Disposal

Decontaminate the instrument before disposal! This luminometer contains electronic parts. To prevent environmental pollution please dispose the instrument and the corresponding accessories according to local legislation. Within the EC dispose the instrument and accessories according to the directive 2002/96/EC or contact our local representative.

This product uses the FreeRTOS.org real time kernel – The freeRTOS.org source code can be obtained by visiting <u>http://www.FreeRTOS.org</u>





Disposal of potential biohazard and chemical waste

To prevent chemical burn, contamination, potential infection and environmental pollution, please dispose chemical or potential biohazard waste always carefully and according to local legislation. It is recommended to treat potential biohazard waste by autoclave.



2.

Getting Started

Carefully unpack the **SIRIUS L Luminometer** and put it on your desktop.

Figure 2-1: Sirius L with 2 injectors



2.1 Luminometer Installation

- □ The instrument should be set up in a dry, dust-free environment and protected from direct sunlight and significant temperature fluctuations!
- Check the package content to be complete for your model.
- ❑ A standard sample holder for 75x12mm and 55x12mm tubes is preinstalled in the factory. Open the measurement chamber and check the sample holder to be still ready installed. Check the 2 screws at the bottom of the sample holder to be tighten.
- □ Sample holder for other sample formats have to be ordered additionally and are not part of the standard scope of delivery.



Figure 2-2:

Sirius L sample holder



- The following sample formats may be used in Sirius L models with and without injectors:
 - With a standard sample holder: 75x12mm tubes 55x12mm tubes (together with the Plexiglas insert)
 - With a microfuge tube sample holder: Microfuge tubes 1.5ml and 2ml



Figure 2-3:

- □ The following sample formats, not allowed for use with injectors, may also be used with a special sample holder. Culture dishes with up to 35mm diameter Scintillation bottles containing up to 20ml Other reaction bottles with up to 12mm diameter
- Do not use the instrument with others than the specified tube formats for your sample holder.
- □ For injector models: Check the injector head and the screws of the injector tubing on top of the instrument to be still tightened to avoid undesirable light incidence. Be careful and make sure that the fittings screws are only finger tightened. Do not use any tools!







- □ Check the tubing connections at the injector pumps to be finger-tightened to avoid leakage.
- Pumps are labeled with numbers to simplify the identification. This number is also used by the PC software to identify the injectors and to avoid confusion.



• Connecting cables at the rear panel:





Connect the instrument to mains with the desktop power supply and the country specific power cord delivered with the instrument. Refer to the power requirements in the Technical Data and to the safety information.



- Connect the instrument to PC via USB or serial port.
- Operating conditions:

Operation Temperature: 10-35°C up to 70% humidity

It is strongly recommended to operate luminescence measurements at room temperature (20°C) to achieve the performance characteristics listed in the technical data.

Operation upon the specified temperature range may cause in a sharp increase of the instrument background to values clearly outside the specified range.

□ Turn the instrument on at the mains switch.



Every time Sirius L is switched on, it acts in a basic mode, called FB12 mode, first. The power LED at the side of the instrument will periodically light up and down. If the instrument is controlled by FB12/ Sirius software V2.0 or higher, it is switched into an advanced mode. The power LED will light up constantly, then. Refer to chapter 4 for details.



Measurement

Overview

3.1

3.



The luminometer should be switched on at least 30 minutes before starting a measurement.

3.1.1 Light Detection

A photomultiplier tube (PMT) is used for light detection. It measures visible light in the spectral range between300 and 630 nm. The PMT converts the photons emitted by the sample into electrical pulses. Every single pulse is then digitally counted (photon counting method). The number of pulses is directly proportional to the light intensity. The light emitted by the sample is collected from the bottom of the tube. To collect as much light as possible, the sample holder is additionally polished to optimize reflection. Sirius L measures a dynamic range of 6 decades.

3.1.2 Units

There is no standardized unit for chemi- or bioluminescence. Therefore, results should only be compared with each other when they are measured on the same instrument. **Berthold Detection Systems** standardizes the Relative Light Units to the number of single photon pulses counted during the period of 1 second (RLU/s), multiplied by the RLU-factor which defaults to a setting to 1.

RLU/s = cps ...RLU factor

The factory-set factor (=1) cannot be changed by the user.

The RLU/s value obtained by the luminometer is the relative light intensity. This does not directly represent the analytical result of an analysis. It is subject to further data evaluation. It is in the liability of the user to evaluate clinical results out of these RLU/s values by use of adequate calculation methods, selected in accordance to the needs of the assay performed. The whole system of luminometer, assay and evaluation software on a PC, must be validated by the user.





Overload

If one or several measured values in the measurement interval exceed the measuring range of the luminometer, OVERLOAD is detected by the instrument. In this case, the shutter will be automatically closed and the software sends the overload signal which is represented by an RLU/s value of 30e6 RLU/s.



Shutter Operation

The Sirius L is equipped with a shutter to protect the photomultiplier tube against light. If the instrument is switched on and the door is closed, the shutter will automatically open. If the user opens the door, the shutter will automatically close and will not open again until the door is closed again. The shutter operation may be identified by the shutter noise. In cases of overload the shutter will always close automatically.

A shutter operation fail is detected by the software. In cases of a not totally opened shutter, the software sends the overload signal to the communication interface.





Shutter and injectors are prepared to be ready for use between "door close" and t=0. All delay times start with t=0.



3.1.5 Sample Tubes

Sample tubes are made of polystyrole (PS) or polyethylene (PE). These materials are sensitive to light and electrostatic influences. Store sample tubes therefore in the dark and protect them from bright light. PS tubes are less sensitive to light than PE tubes. Please avoid also immoderate tube handling to prohibit electrostatic influences, which may deflect the reagents during injection and result in splashes at the inner walls of the tubes.

3.2 Start a Measurement

Sirius L is fully computer operated. The luminometer has to be connected to a PC via serial or USB cable. Refer to the chapter 4 for installation and set up of the software. Select a protocol type, set the parameters and run the protocol for measurement.

To start a measurement, open the sample drawer and insert the first tube into the sample holder. Close the measurement chamber. The measurement will start immediately after "door close" and continue until the measurement is finished. Open the door to change the sample tube and close it again to start the next measurement.

3.3 Instrument Background Measurement

It is recommended to perform instrument background measurements without tube daily, after the instrument has warmed up for 30 minutes, to make sure, that the instrument works well.

Please refer, too, to information about Quality Control in chapter 1.6 and PC software in chapter 4.



Instrument Background Measurement:

□ Use the PC software and select a Single Assay protocol with the following parameters:

Delay time: 1s Measurement time: 10s Start measurement by: Door close Number of Replicates: 10 Number of Samples: 1 First sample is background: No Injectors: not active

- □ Perform the measurements without tubes.
- □ The instrument background meets the specifications if the following values are obtained:

Background average: #100 RLU/s A single background value: #130 RLU/s

□ In cases of fail repeat the measurement and refer to the troubleshooting list.

3.4 Measurement with Injectors

3.4.1 Reflectors



The standard sample holder and the microfuge tube sample holder are suitable for use with injectors. The standard sample holder can accommodate 12mm tubes with a length of 75 and 55 mm. For the 55mm tubes, the Plexiglas insert (included with the shipment) has to be inserted from below. The microfuge tube sample holder accommodates 1.5ml and 2ml microfuge tubes with caps.



3.4.2 Sample Presence Detector

Start up of Injectors

2. too.

The Sirius luminometer contains a sample presence detector to prevent injection into an empty sample holder. In cases of a missing sample tube, a protocol with injection cannot be operated and the user is informed by the software to insert a sample tube for operation. The sample detection is only active in cases of activated injectors.

3.4.3





Depending on the order, the configuration will consist of 0, 1 or 2 injector pumps installed in the instrument. Pumps are labeled with numbers to simplify the identification. This number is also used by the PC software to identify the injectors and to avoid confusion. Refer to figure 2-4 in chapter

- □ Place the reagent bottles into the reagent holders.
- □ Connect the reagent bottles to the white tubing of the respective injector pump. Check afterwards that reagent bottles have been connected to the appropriate pump and have not been mixed up.
- Assay components may cause a biohazard or risk of infection. Always handle the injection lines carefully and refer to the kit inserts and the safety instructions and precautions prior to work.
- □ All operations and measurements are controlled by the software.
- □ For priming of injectors please refer to chapter 4.4 for detailed instructions.
- ❑ All parts of the injector system coming in contact with reagents are chemically inert and can be used for a longer period of time. They have to be cleaned periodically according to the description in chapter 11.2. The injector lines and tips should be replaced once a year.



3.4.4 Removing the Injector Head

Do not unscrew and remove the injector head!

The injector head should only be removed in cases of downgrade of the injectors. Contact **Berthold Detection Systems** for further instructions. For replacement of the injector tips, located in the injector head, refer additionally to chapter 11.8.



In cases of continued work with a mounted blind screw instead of injector head (downgrade of an injector instrument): Please do not touch the blind screw during measurement. A strong electrostatic discharge could cause light within the measurement chamber.



4.

Working with PC Software

Sirius L is fully computer operated. Depending on the Software version controlling the luminometer, it acts in two different measurement modes.

Advanced mode: Every time Sirius L is switched on, it acts in a unidirectional basic mode, called FB12 mode, first. If Sirius L is operated via FB12/ Sirius software V2.0 and higher, the software switches the luminometer from the basic FB12 mode to a bidirectional advanced mode. All operations including injector functions and shutter operation are now controlled by the software. Additionally the protocol types **Dual Assay** and **Single Kinetic** have been revised for the advanced mode, as described in the respective chapters.

FB12 mode: FB12/ Sirius Software versions V1.5 and lower operate Sirius L in a unidirectional basic mode, called FB12 mode. Once the Sirius L is switched on, it sends data as photon counts per second periodically every 200ms via RS232 or USB interface. The data can be integrated using PC software for any timeframe multiple of 200ms. The injectors are not controlled and operated by these software versions. The shutter operation is controlled by firmware.

4.1 PC Software Installation

Important information

Plea

Please ensure the software is installed in administrator mode. For work with the software administrator rights are not necessary.

- Close all *Windows* applications.
- □ Insert software CD in the CD drive. The CD navigator will automatically start the internet browser.
- □ Select "Software" in the navigator bar, then "FB12/Sirius Software Installation".
- Double-click the **Setup.exe** file to initiate the setup.



- □ If the CD navigator does not automatically start, open Windows Explorer and select CD drive. Double-click **start.htm** to start the CD and continue, as described above, to install the software.
- Follow the instructions of the setup program. When the program is started, the [Welcome] screen is displayed. Click <Next>. The [Choose Destination Location] dialog box appears. Click <Next> to keep the defaulted directory C:\...\FB12 software. Click <Browse> to choose another directory. Select the base software and the different protocol types in the [Select Components] dialog box.
- Click <Next>: [Select Program Manager Group].
- □ Click <**Next**> (display of [**Start Installation**]).
- □ Click **<Next>** to start the installation.
- Upon completion of the installation process, click <**Finish**>.
- □ The software includes a 30 days trial option for nonpurchased software components.

Installation of additional protocol types

Proceed as described above. Select the protocol types needed in the [**Software Components**] dialog box. Then complete the installation.

Instructions for use of USB connection

USB connection is recommended for operating systems Windows XP and higher. For Windows 2000 please use serial connection only!

After installation of the FB12/ Sirius software, connect the luminometer to PC using the USB.

- □ The PC will recognize that there is a new device and ask for a driver. The USB driver is part of the FB12/Sirius software and stored in the directory C:\....\Berthold Detection Systems\FB12 Software V2\Driver\SiriusL\ USB. Follow the installation wizard and insert this path to install the driver.
- Depending on your PC and its operating system, the installation wizard for the driver software may not occur automatically. Please refer to the Technical Note for USB driver installation, coming with the instrument, too.



4.2 Initial Software Start Up

- Double-click on the **FB12** software ICON.
- □ Click on the <**Options**> button and press <**Find luminometer**>. The PC software searches the COM port where the luminometer is connected, automatically.
- □ In the [Default Data storage directory] box, enter the directory where the measurement data are to be saved. It is recommended to use the default storage directory.

Important information for Windows Vista and Win 7 users:

The operating system stores measurement data to the folder <My documents> automatically, independent from the entered data storage directory.

- □ To hear a signal at the end of a measurement, select the item [**Beep when measurement is completed**]. For Win 7 this item is only available, if additional loudspeakers are installed.
- □ Click <**OK**> to save the entries. The program changes to the Protocol Manager.



Software Registration

FB12/ Sirius software may be used for 30 days without registration. A prompt to enter a registration password will appear each time a protocol is selected or the measurement menu is opened until registration passwords are entered. User may close the **Registration Form** dialog box by clicking **<Run Now, Register Later>** and continue working with the software.

The passwords must be entered within 30 days in order to continue working with the software. The [**Registration Form**] dialog box appears again; either enter registration password or click **Cancel**> to close this dialog box.

Passwords may be requested via **E-Mail, Fax or Web**. Follow the instructions on the registration form. Press the respective E-mail, Web or Fax button, insert required information in the predefined mail, fax form or web registration form and forward to **Berthold Detection Systems.**

Figure 4-1:	Registratio	n Form	×
[Registration		Single Assay For FB12	2/Sirius Software
Formj dialog box	Cap	right (c) 2005 Berthold Detection Sys	tens GmbH, Al Rights Reserved
	The	rregistered version of the Single Assu Please register before that time (ay will nun for 30 days after hist nun to enable unlimited uze
	Diays left	Trial Period Expired	Parties equip last.
`	System ID	FBS-135653	
	To obtain the Press the e-ru information Or visit http:/	password to enable the software, ple nail, the web or the fax button to get th /www.berthold-ds.com/register/a1.ph	ease contact Betthold Detection Systems, re contact formular and insett all required ID
\longrightarrow	E-mail	Web	Fax
	Please order	your registration information here	
	Name		
	Company:	Ĩ.	
	Password	l.	
			Court 1
	1 i i	Heguter Now	Carcei

Passwords may also be requested via the BDS homepage. Please provide the **System ID** of the PC. The number is printed in red in the registration dialog of the software.



Registration

Owner will receive registration passwords from **Berthold Detection Systems** (one password for each purchased protocol). When [**Registration Form**] dialog box appears, enter the user's name, the company name and the **password for that protocol**. Click **<Register Now>**. Upon entry of correct passwords, the software may continue to be used. The [**Registration Form**] dialog box will not appear again.

Important information for Windows Vista and Win 7 user:

Due to limited writing authorizations the registration may occur temporary delayed. It is rarely possible that the registration of a single protocol has to be repeated.



4.3 Basic Software Structure





4.3.1 Protocol Manager

[Protocol types]

Shows the protocol types which can be used as templates for creating protocols.

[Runable protocols]

Shows a list of created protocols. Protocols are edited using the buttons in this box:

<Run>

Shows the measurement menu and you can start a measurement with the selected protocol.

<Edit>

Edit the parameters in the selected protocol.

<Delete>

Delete the selected protocol.

Quick Measurement

is not a protocol type, but a measurement protocol; parameters may be changed, but may not be stored in separate protocols.

<Retrieve>

Shows the stored measurement data; double-click to open the desired data file (*.MDS).

<Priming Injectors>

Opens the Priming dialog. Injectors have to be primed prior to use.

<Options>

Gives information about the used COM port, the Baud rate and the default data storage directory.

<About>

Gives information about software version number.

<Close>

Closes the protocol manager.



4.3.2 Measurement Menu

Select the desired protocol and click <**Run**>. The measurement menu is displayed.

Measurement start

By opening / closing the sample drawer or pressing the green button or the spacebar.

Meas. completed

As soon as the measurement time is over.

Measurement stop

By opening the sample drawer or pressing the red button.

Result fields

To change the size of a result field, move cursor to the right-hand margin of the header. As soon as the cursor shape changes to a double-arrow, click the left mouse button and drag the cursor, with the mouse button held down, left or right.

\mathbf{x}	

EXCEL Transfer

Click the button depicted to the left to manually import data into a new Excel worksheet. The transfer may also be automated in the protocol.

Working with the Software 4.3.3

Tool buttons on the measurement menu simplify program handling. Apart from a few exceptions you need not select any pull-down menus.

Tool buttons and their meaning:

Button	Meaning	Pull-down menu -> Option
	Save measurement data	[File] -> [Save (as)]
×	Import measurement data into Excel	[Edit] ->[Send to Excel]
<u>e</u>	Print	[File] -> [Print]
	Start measurement	[Measure] -> [Start Measurement]
O	Stop measurement	[Measure] -> [Stop Measurement]
×	Delete last measurement	[Measure] -> [Delete Last Measurement]
	Display results in spreadsheet	[Measure] -> [Kinetics]
	Display results in a graph	[Measure] -> [Kinetics]
Ξ	Tile window horizontally	[Window] -> [Tile Horizontal]
	Tile window vertically	[Window] -> [Tile Vertical]
D	Create new data file in measurement menu	
E	Copy selected data to clipboard	



4.4 Priming Injectors

Injector tubing has to be washed with 5ml of distilled water prior to first use. Wash also before starting a measurement and when changing reagents. Proceed as described below.

For measurement injections, the tubing has to be primed first to ensure that the full volume is injected with the first shot.

To wash or prime the tubing, insert an empty sample tube into the sample holder!

The dead volume of the tubing is lower than 700µl.

Please note:



Depending on the sample holder and the sample tubes selected, the total volume for priming is limited. Prime the injectors consecutively and check the total priming volume to be lower than the capacity of your sample tubes to avoid overflow. Prime every injector separately into a new and empty tube.

Proceed as follows to wash and prime the tubing:

- Connect the reagent bottles to the white tubing of the respective injector pump. The numbers of the injectors used in the software are in accordance with the numbers on the pumps.
- □ Check afterwards that reagent bottles have been connected to the appropriate pump and have not been mixed up.
- □ Select <priming injectors> in the protocol manager. The priming dialog will open.



Figure 4-3: Priming dialog

Priming injec	ctors			
🔽 Injector 1	Direction to Tube	Volume [μl]	Strokes	Total Volume [μl]
☐ Injector 2	to Tube	300 × X	3 *	900
	Prime Cano	el	Cl <u>o</u> se	

- □ Mark the check box to enable the respective injector and the list boxes [Direction], [Volume [µl]], and [Strokes].
- □ Set the injection [Direction]. Select between [to Tube] and [to Bottle]. It is recommended to select [to Tube].
- □ Set the **[Volume]** of a single stroke.
- □ Select the number of **[Strokes]** to define the total injection volume:

[Volume] x [Strokes] = [Total volume]

- □ To avoid leavings of air, reagents or water in the injector system, the **[Total Volume]** should not be lower than 750µl. It is recommended to operate a second priming cycle with a new empty tube for every injector, to make sure that residua have been eliminated.
- Recommended maximum total volume (µl) for priming of a single injector:

75x12mm tubes : ≤3000µl 55x12mm tubes: ≤2000µl Microfuge tubes 2ml: ≤1000µl Microfuge tubes 1,5ml: ≤750µl

- □ Click on **<Prime>**. Then the selected injectors are initialized and the lines are washed/primed. The injectors are now available for measurement.
- □ Click on<**Close>** to return to the protocol manager.

4.5 Total Reaction Volume



Sirius L enables the user to inject into different sample formats. Before preparing a measurement protocol, please check that the total reaction volume is within the volume range of the selected tube. Especially when microfuge tubes are used, the sample and injection volume have to be reduced to avoid overflow and splashes outside the tube.

Recommended maximum total reaction volume:



75x12mm tubes : ≤3000µl 55x12mm tubes: ≤2000µl Microfuge tubes 2ml: ≤1000µl Microfuge tubes 1,5ml: ≤750µl

For all protocol types:

Please set the parameter **[volume]** of the injectors in your protocol in accordance to this recommendation. If necessary adapt your assay accordingly.


4.6 Comparison of Software Protocols

To help you get a quick overview of the different protocol types, we have summarized their functions in the table below:

	Quick Measur.	Single Assay	Single Kinetics	Multiple Kinetics	Dual Assay	Cut-Off- Assay
Function		Measurement of several samples with replicates	Continuous measurement of one sample	Several samples, discontinuous measurement	Measurement of sample series A and B	Classification of samples
1 st sample = BG	no	yes	no BG may be defined	no BG may be defined	yes	Yes
Replicates	no	flexible	yes	yes	same number for A and B	yes: for samples, neg. and pos. controls
Number of samples	any	flexible	1 sample measured with any data points	any, with any data points	same number for A and B	yes: for samples, neg. and pos. controls
Calculations	none	>1 replicate: average value %stdv/av	>1 replicate: average value %stdv/av repl. 1, repl. 2 log/normal scaling, smoothing, peak half-life time during increase and decay	>1 replicate: average value %stdv/av repl. 1, repl. 2 log/normal scaling, smoothing, peak half-life time during increase and decay	>1 replicate: average value %stdv/av	>1 replicate: average value %stdv/av
Kinetics graph	none	Not displayed in advanced mode	complete graph of all replicates	complete graph of all replicates	Not displayed in advanced mode	Not displayed in advanced mode
Miscellaneous	Meas. sequence	predefined sample names		measurement start only at closing of sample drawer	predefined sample names different calculation formulas for A and B	predefined sample names different cut-off calculations and classifications

.....

BG = Background

5. Quick Measurement

This protocol type measures the raw data of consecutively numbered samples.

5.1 Protocol

- In the Protocol Manager, select the [Quick Measurement] protocol and click <Edit>. The [Quick Measurement Parameters] dialog box appears.
- □ Set **Delay time** for the measurement and **Measurement time** by clicking on the respective arrow buttons.
- □ Select **Injectors** by marking the respective check box and set injector **Volume** and **Delay** time.
- □ Click <**Save**> to save the measurement parameters. The program returns to the Protocol Manager.

5.2 Measurements

- □ In the **Protocol Manager**, select the [**Quick Measurement**] protocol and click <**Run**>. The measurement menu appears showing the parameters defined for injection and measurement.
- Open sample drawer and insert sample tube.
- □ Close sample drawer. The measurement process starts as soon as the preset delay time is over.
- □ The result is displayed at the end of the measurement.
- □ Proceed in the same manner to measure the next sample.



6. Single Assay

This protocol type allows you to run background measurements and to measure replicates of samples.

6.1 Protocol

Figure 6-1:

Dialog box [Single Assay

Page 1

Parameters]

Select Single Assay and push <Create>.

Single Assay Parameters - Single Assay Measure and delay times Measurement time: Delay time: 1.0s 3.0s Save As... Start measurement by Cancel Door close Start button or space bar or enter key Number of replicates and samples Number of replicates: Number of samples: 2 ÷ 1 • First sample is background Injectors #1 Volume [ul] #2 Volume [ul] Delay [s] Delay [s] ☑ 100 ☑ 100 1 2 Comments: Advanced Options... Excel (TM) Options...

[Delay time]

Delay before measurement; can be set only if a measurement is started by closing the sample drawer.

[Measurement time]

Measurement time for each sample measurement.



[Start Meas. by]

Select how to start the measurement on the measurement menu:

[Start button]

By clicking the Start button or pressing the spacebar. No delay time can be defined here!

[Door close]

By closing the sample drawer of the luminometer.

[Number of replicates]

[Number of samples]

[**First sample is background**] Measurements of the first sample replicate are considered background measurements.

[Injectors]

Injectors are only active if the check box is marked. Selection of the respective injector by marking check box #1 and/or #2.

[Volume]

Volume to be injected by the respective injector.

[Delay]

Delay before injection.

<Advanced Options>

Opens page 2 of the Single Assay Parameters dialog box.

[Grid Options]

You may select a flexible table set-up, so that the number of replicates and/or samples may be changed during measurement at the push of a button.

[Measurement orders]

Order of measurement

[Across]

Measurements are carried out by rows: first, all replicates of the first sample, then all those of the second sample, etc.

[Down]

Measurements are done by columns, e.g. first the first replicate of all samples, then the second replicate of all samples, etc.

Sample	Rep1	Rep2
1		
2	-	
3		
Sample	Rep1	Rep2
Sample 1	Rep1	Rep2

3



6.2 Measurements

Preparation Select a protocol (type **Single Assay**), click **<Run>** and the program will go to the measurement menu.

Parameter Display of the run parameters. If you have defined the number of replicates as variable, you may change this by clicking on the respective button. A background value can also be set manually if no background measurement is carried out. The value entered here is subtracted from the raw data of each sample.

Measurement start By opening / closing the sample drawer or by clicking on the Start button.

Results The measurement values will be displayed in a table depending on the definition in the protocol with the following columns (one row for each sample):

[Sample] Sample name.

- [**Rep...**] One column has been reserved for each replicate. If the number of replicates was defined as variable in the protocol, you may increase the number of columns by clicking the appropriate button (+).
- [Average] This column contains the average values, calculated from the replicate results of that row.
- [%SD/Avg] Standard deviation in percent, divided by average value.
- [**Net**] Average value minus background value.
- [%SD/Net] Standard deviation in percent, divided by net average value.

Graph

The measurement data trend of each single measurement with data points can be displayed in a graph by clicking the graph button. The data will be transferred to the graph at the end of every measurement.



7. Dual Assay

In the **Dual Assay** mode two sample series A and B are measured. The sample series can either be measured in the order A, B, A, B or A, A, B, B. Each series may start with an injection. A mathematical ratio may be calculated between both measurement series.

Typical applications: Dual Luciferase Reporter Gene Assay

i

Measurement order:

A, B, A, B, A, B

Description only valid for Sirius L with FB12/ Sirius software V2.0 or higher.

If the **DUAL MEASUREMENT** Protocol is selected, the measurement sequence is automatically divided into two cycles; an injection may be performed in each cycle. The delay times for measurements and injections are calculated <u>starting</u> with the cycle 1.



Figure 7-1: Calculation of delay and measurement times





For measurement order A,B,A,B,A,B cycle 2 is always started automatically.

If only <u>one</u> injector is installed (**Injector 1**), but you would like to perform injections in **cycle 2**, set the delay time for **Injector 1** so high that it extends into **cycle 2**.

Measurement order: A, A, A; B, B, B



Figure 7-2:

[Dual Assay

Parameters]

Dialog box

Page 1

7.1

The measurement sequence A,A,A,...,B,B,B in the protocol **DUAL ASSAY** may be considered as <u>two individual runs carried out in</u> <u>succession. This applies for the injector operation, too.</u> The respective injector parameters will be carried out in sequence A and sequence B in succession. The PC software measures and calculates the results as defined in the PC protocol.

Protocol

Select Dual Assay and press <Create>.

Lange Subtraction State of the second Save Series A measurement and delay times Series B measurement and delay times Delay time: Measurement time: Delay time: Measurement time: Save As... • 10.0s • 10.0s 3.0s ÷ 16.0s ÷ Cancel Start measurement by Number of replicates and samples Start button or space bar or enter key Number of replicates: Number of samples: 3 \exists 5 ÷ Door close Measurement options Measurement order: Transform: First sample is background A/B A, B, A, B, ... -Ŧ Injectors #2 Volume [ul] #1 Volume [ul] Delay [s] Delay [s] 100 ÷ ÷ 1 100 ÷ 14 Comments: Excel (TM) Options... Advanced Options...

[Series A measurement and delay time]

[Delay time]

Delay for sequence A prior to each measurement; can be set only if a measurement is started by closing the sample drawer.



[Measurement time] for each sample measurement of series A.

[Series B measurement and delay time]

Enter parameters for series B in the same manner as for series A.

[Start Meas. by]

Select how to start the measurement:

[Start button]

By clicking on the Start button or by *pressing* the spacebar. No delay time can be defined here!

[Door close]

By closing the sample drawer.

[Number of replicates and samples]

[Number of replicates]

[Number of samples]

[First sample is background]

Measurements of the first sample replicate are considered background measurements.

[Measurement options]

[Transform]

Select the calculation formula.

[Measurement order]

Selection of order: Either one sample of sequence B after each one of sequence A, or all samples of sequence A in a row and then all those of sequence B.

[Injectors]

Injectors are only active if the check box is marked. Selection of the respective injector by marking check box #1 and/or #2.

[Volume]

Volume to be injected by the respective injector.

[Delay]

Delay before injection.



7.2 Measurements

Preparation Select a protocol (type **Dual Assay**), click <**Run**> and the program will go to the measurement menu.

- Parameter Display of protocol parameters. The measured background values are displayed in the [Background A] and [Background B] text boxes, if the first sample of each sequence was defined as background in the protocol. A background value can be entered manually. The value displayed here will be subtracted from the raw data of each sample.
- Measurement start By opening / closing the sample drawer or by clicking the Start button.

Results Display of measured values:

[**Sample**] Sample name.

- [Net A] Net rate of series A = average value of the replicates of the first sample of sequence A, minus background.
 [Net B] Net rate of series B
- [A...B] Calculation of the two net rates according to the formula defined in the protocol
- [%SD/Avg] Standard deviation in percent/average value.
- [**Rep ...A**] Measurement value for replicates of series A.
- [Average A] Average value from replicate results of series A of the respective row.
- [%SD/Avg] Standard deviation in percent/average value.
- [Net] Average value minus background (series A).
- [%SD/Net] Standard deviation in percent/average value.

The following columns contain the measured values for series B.



8. Single Kinetics

The measurement type **Single Kinetics** is used to measure the trend of the light emission over a specific period of time.

i

Figure 8-1: Dialog box [Dual Assay Parameters]

Page 1

8.1

Description only valid for Sirius L with FB12/ Sirius software V2.0 or higher.

Protocol

Select Single Kinetics and push <Create>.

Single Kinetics Parameters - Single Kinetics	
Time settings Delay time: 1.0s 10.00s 10.00s 10.00s 10.00s	Save As Cancel
Note: Sampling time = Total duration / No. of data points (1ms99ms or multiples of 100ms up to 10 min) Start timer by No. of replicates:	
Start button or space bar or enter key O Door close Injectors	
#1 Volume [ul] Delay [s] #2 Volume [ul] Delay [s] Image: Comment: 100 0 100	
Advanced Options Excel (TM) Options	

[Time settings]

The following 4 parameters of this group box are mutually conditional (with the exception of delay time):

[Delay time]

Delay before each measurement; can be set only if a measurement is started by closing the sample drawer.

[Total duration]

Total sample measurement time, including the following times:



[Sampling time]

Measurement time per data point.

[No. of Data points]

Number of data points multiplied by the sampling time shows the total duration of the measurement.



Select the **[Total duration]** first and the **[Sampling time]** afterwards. The **[No. of Data points]** is the result of these two settings and will be calculated automatically.

For [**Sampling time**] lower than 0,1s the [**No. of Data points**] is limited to 250 points.

[Start Meas. by]

Select how measurement is to be started on the measurement menu.

[Start button]

By clicking the Start button or pressing the spacebar. No delay time can be defined here!

[Door close]

By closing the sample drawer. Measurements with injectors can only be started in this manner.

[Number of replicates]

[Injectors]

Injectors are only active if the check box is marked. Selection of the respective injector by marking check box #1 and/or #2.

[Volume]

Volume to be injected by the respective injector.

[**Delay**] Delay before injection.

<Advanced Options>

Click this button to open page 2 of the **Single Kinetics Parameters** dialog box.

[Use luminometer as time basis]

Select the luminometer as time basis.



8.2	Measurements		
Preparation	Select a protocol (type Single Kinetics), click <run></run> and the program will go to the measurement menu.		
Parameter	Display of protocol parameters. You may type a background value into the [Background RLU/s] box. The value entered here is subtracted from the raw data of each sample.		
Measurement start	By opening/closing the sample drawer or by clicking the Start button.		
Data transfer	For protocols with a sampling time < 0,1s: All measured data of a replicate will be transferred to the result table at once at the end of the measurement.		
	For protocols with a sampling time ≥0,1s: The measured value for every sampling time interval will be transferred immediately after measurement.		
Results The measurement values will be displayed in a t on the definition in the protocol with the following row for each sample):		ent values will be displayed in a table depending n in the protocol with the following columns (one mple):	
	[Time]	Length of time the light emission is measured.	
	[Rep]	Measurement values for individual replicates.	
	[Average]	Average value of replicates.	
	[%stdv/Av]	Standard deviation in percent, divided by average value.	
	[Net]	Net value (average value minus BG value)	
	[%SD/Net]	Standard deviation in percent, divided by net average value.	
Graph	The measurement displayed in a measurement, processing the window.	ent data trend of each single measurement can be a graph, either online or at the end of the by clicking the graph button. Further options for graph become visible when you enlarge the graph	
	[Group]	Select which replicate curve(s) is to be displayed.	
	[Smoothing]	Smoothes all displayed curves.	
	[Log scale]	Logarithmic scaling.	
	[RLU-range]	Select if scaling is to be applied automatically or manually.	



Multiple Kinetics 9.

In the Multiple Kinetics mode, the trend of several samples is measured in parallel over a longer period of time.

9.1 **Protocol**

Figure 9-1: Dialog box

Select Multiple Kinetics and push <Create>.

Figure 9-1:	🕅 Multiple Kinetics Parameters - Multiple Kinetics
Dialog box [Multiple Kinetics Parameters]	Time settings Delay time: Total duration: Sampling time: 1.0s • 1m 0.0s • Save As
Page 1	No. of data points: Interval time: 6 10.0s Cancel Note: Interval time = Total duration / No. of data points (to nearest 0.2s)
	Replicates and samples Number of replicates: Number of samples: 1 2 1 2 Injectors #2 #1 Volume [ul] Delay [s] #2 200 0
	Comments
	Excel (TM) Options

[Time settings]

The following 5 parameters of this group box are mutually conditional (with the exception of delay time):

[Delay time]

Delay before every measurement.

[Total duration]

Total sample measurement time, including the following times:

[Sampling time]

Measurement time per data point.



[No. of Data points]

Number of data points multiplied by the interval time shows the total duration of the measurement.

[Interval time]

The interval time may be longer than the respective measurement time of the data point.

[Number of replicates / samples]

The number of replicates or samples.



[Injectors]

Injectors are only active if the check box is marked. Selection of the respective injector by marking check box #1 and/or #2. Injections can only take place once in the first measurement cycle of a tube according to the injection delay time adjusted. They are not repeated in every measurement interval.

[Volume]

Volume to be injected by the respective injector.

[Delay]

Delay before injection.

A measurement can only be started by closing the sample drawer !

9.2 Measurements

- **Preparation** Select a protocol (type **Multiple** *Kinetics)*, click <*Run*> and the program will go to the measurement menu.
- ParameterDisplay of protocol parameters. You may type a background value
into the [Background RLU/s] box. The value entered here is
subtracted from the raw data of each sample.

Measurement start By opening/closing the sample drawer.



Results	Measured va	lues are displayed in a table:
	[Time]	Length of time, over which the light emission is measured. The number of data points in [time] is calculated by dividing the total measurement duration by the interval time.
	[S1 Rep1]	Measurement value of the first replicate of the first sample.
	[S1 Rep2]	Measurement value of the second replicate of the first sample.
	[Average]	Average value of replicates of the first sample.
	[%SD/Avg]	Standard deviation in percent, divided by average value.
	[Net]	Net value (average value minus BG value).
	[%SD/Net]	Standard deviation in percent, divided by net average value.
	[S2 Rep1]	Measurement value of the first replicate of the second sample.
	[S2 Rep2]	Measurement value of the second replicate of the second sample.
	[Average]	Average value of replicates of the second sample.
	[%SD/avg]	Standard deviation in percent, divided by average value.
	[Net]	Net value (average value minus BG value).
	[%SD/Net]	Standard deviation in percent, divided by net average value.
	[SRep]	Further samples with replicates.
Graph	The measure	ement data trend of each single measurement can be

The measurement data trend of each single measurement can be displayed in a graph, either online or at the end of the measurement, by clicking the graph button. Further options for processing the graph become visible when you enlarge the graph window.

10. Cut-off Assay

A **Cut-off** protocol classifies samples into three categories. Socalled cut-off thresholds (high and low) are measured by defining fixed parameters and measuring negative and positive controls.

10.1 Protocol

Cut-off Assay Parameters - Cut-off Assay Measure and delay times Delay time: Measurement time: Save As... 3.0s 1.0s -Start measurement by Cancel Start button or space Door close bar or enter key Negative/Positive controls Analytes Negative replicates: Positive replicates: Replicates: Samples: ÷ 1 ÷ 1 1 1 First sample is background. Cut-off calculations Offset Neg. Factor Pos. Factor + 1,00 0,00 Low cut-off = 0 * (Neg. Net) + * (Pos. Net) Offset Neg. Factor Pos. Factor * (Pos. Net) High cut-off = 0 + 0,00 * (Neg. Net) + 1,00 Injectors #1 Volume [ul] #2 Volume [ul] Delay [s] Delay [s] ~ 100 100 2 1 Comments: v Advanced Options... Excel (TM) Options...

Select Cut-off ASSAY and press <Create>.

Figure 10-1: Dialog box [Cut-off Parameters]

Page 1



[Delay time]

Delay time before measurement; can be set only if measurement is started by closing the sample drawer.

[Measur. time]

Measurement time for each sample measurement.

[Start Meas. by]

Select how to start the measurement on the measurement menu:

[Start button]

By clicking the Start button or pressing the spacebar. No delay time can be defined here!

[Door close]

By closing the sample drawer of the luminometer.

[Analytes] Define the number of replicates and samples.

[Negative/Positive controls]

Define the number of replicates of negative and positive controls.

[First sample is background]

Measurements of the first sample replicates are treated as background measurements.

[Cut-off calculations]

Definition of low and high threshold by the following parameters:

Offset (RLU) value

[Offset]

- + [Neg. Factor] any constant
- x [Neg. Net] net rate of negative control
- + [Pos. Factor] any constant
- x [Pos. Net] net rate of positive control

[Injectors]

Injectors are only active if the check box is marked. Selection of the respective injector by marking check box #1 and/or #2.



[Volume]

Volume to be injected by the respective injector.

[Delay]

Delay before injection.

<Advanced Options>

Click this button to open page 2 of the **Cut-off Parameters** dialog box.

[Result text]

Designation for Cut-off results. The following designations are defaulted:

[Negative result text]	NEG
[Positive result text]	POS
[Equivocal result text]	+/-

10.2 Measurements

PreparationSelect a protocol (type Cut-off), click <Run> and the program will
go to the measurement menu.

ParameterDisplay of protocol parameters. You may type a background value
into the [Background RLU/s] box. The value entered here is
subtracted from the raw data of each sample.

Measurement start By opening/closing the sample drawer or by clicking the Start button.

Results Measured values are displayed in a table:

[Sample]	Sample name.
[Rep]	A column is reserved for each replicate.
[Result]	Result column. POS, NEG or +/- as result.
[Average]	Average value from the replicates of one sample.
[%SD/Avg]	Standard deviation in percent/average value
[Net]	Average value minus background value.
[%SD/Net]	Standard deviation in percent/net average value.



11. Maintenance

The **SIRIUS L Tube Luminometer** hardly requires any maintenance. It should, however, be protected from dirt and cleaned occasionally. Use only the described instructions for cleaning and decontamination.

Surfaces of Sirius L:

Housing:	Aluminium, varnished
Door:	PUR-Baydur 110F, varnished
Sample holder:	Aluminium, outside black eloxadized

The surfaces of Sirius L are tested to be resistant against the chemical solutions for cleaning and decontamination.

Components of the Injector system:

Tubing:	PTFE (Teflon)
Fittings:	PTFE (Teflon)
Nuts, T-Piece:	Kel-F
Pumps:	PTFE, Kel-F, glass

The injector system is composed of well-proven and chemically inert material, resistant against the solutions for cleaning and decontamination of the injector system.

11.1 Maintenance of Surfaces

Cleaning of surfaces

The instrument surface is protected by a robust and washable varnish that can be cleaned using a damp cloth. If necessary, use a mild detergent. The sample drawer, the sample holder and especially the measurement window also have to be kept clean and should be wiped off with a damp cloth. If necessary, use a mild detergent or 70% Isopropanol. A periodic weekly cleaning is recommended. Additionally, the measurement chamber and sample holder have to be cleaned in cases of unexpected high measurement values.



Decontamination of surfaces

In cases of biohazard spillage, other kinds of pollution or reshipment for service, decontamination is necessary. The outer surface, the measurement chamber and the sample holder may be decontaminated with 70% Ethanol or 70% Isopropanol or 0,5% Na-Hypochlorite solution (containing 5% free Chlorine). Always wear gloves while handling with chemical solutions. Follow also the instructions and recommendations included in the package insert for the test to be conducted. After use of Na-Hypochlorite solution, wipe off the respective surfaces with distilled water and dry them afterwards to avoid corrosion.



No fluid should ever enter the instrument! If this happens, disconnect the device from mains and call for service!

Before Service or shipping any instrument back to **Berthold Detection Systems**, the instrument has to be decontaminated. Refer to the Decontamination Form in this manual for details.

11.2 Maintenance of the Injector System

All materials coming in contact with reagents are chemically inert and can be used repeatedly for a longer period of time. Anyway, regular maintenance is necessary.

Rinsing the injector system (see section 11.4).

Daily	Rinse with deionized or distilled water
Before and after	Rinse using acid/base cleaning procedure. See chapter 11.5.2.
longer breaks	

Regular Maintenance and Checks

Injector pump	Rinse after use.
	• If the pump has not been in use for a longer period of time,
	you should clean it with water.
	 Do not operate it without liquid.
	Check for leaks. If you detect any leaks, call service.
Injector tubing	 Do not bend the reagent lines!
	• Check tubing and screw fittings for leaks. If you detect any
	leaks, replace the injector tubing (see enclosed spare part kit).



Injector tips	Replace injector tips regularly.
Injector unit	• If liquid gets inside the instruments, turn the instrument off,
	open the injector unit and clean the dirty parts. Try to identify
	the cause of the leak (leaky screw fitting or faulty pumps) and
	take the appropriate measures (tighten fitting screw or replace
	injector unit together with the tubing).

Decontamination of injector system

In cases of potential biohazard contamination	 Rinse the injector system with distilled water first. Decontaminate the injector system according to description in chapter 11.6.
Storage/Return	 Decontaminate the luminometer. See and fill out the Decontamination form

11.3 Maintenance Frequency Schedule

	Daily/ before/after use	Weekly/ As needed	Yearly/ As needed	Before Storage/Ship ment/As needed
Tasks for all mode	ls			
Clean external surface		\checkmark		
Clean sample compartment		\checkmark		
Decontaminate instrument surfaces				\checkmark
Additional Tasks for Injector Models				
Rinse injector pump and tubing	\checkmark			
Clean injector head				
Exchange injector tips				

Clean reagent		
bottles		
Replace injector		
tubing		
Rinse injector		
system with		 \checkmark
acid/base		
Decontaminate		
injector system		



Make sure no liquid gets inside the instrument! If this happens, disconnect the device from mains and call for service!

11.4 Rinsing/Priming Injector Tubing

Rinsing and priming of the injector tubing is controlled by the PC software. Use the submenu **<Priming Injectors**>(see chapter 4.3.1).

Rinse the injector tubing system regularly after use with distilled water.

11.5 Cleaning the Injector System

The injector system has to be cleaned regularly to keep it fully functional and to avoid wrong measurement results due to reagent leavings or blocked reagent lines. Use the **submenu <Priming Injectors>** of the PC software to clean the injector system.

11.5.1 Daily Cleaning Procedure

Use distilled water to flush reagents out of the injector system. Put the still filled tubing into the supply bottle containing distilled water. Open the priming dialog and act according to the description in chapter 4.4Select the maximum total priming volume recommended for your sample format.



11.5.2 Basic Cleaning Before or After Long Breaks

Acid/Base Cleaning Procedure

To retain the fluids in the pumps for approximately 10 minutes, open the priming dialog and act according to the description in chapter 4.4. Select the maximum total priming volume recommended for your sample format

Place a new sample tube into the instrument for each cycle!

Proceed as follows:

1. Wash with distilled water

□ Proceed as described above.

2. First cleaning step

- □ Fill the respective injector with **0.1N NaOH**.
- □ Leave this solution in the system for approximately 10 minutes.
- **□** Rinse the injector system with distilled water.
- 3. Second cleaning step
- □ Fill the respective injector with **0.1N HCL**.
- □ Leave this solution in the system for approximately 10 minutes.
- □ Rinse the injector system with distilled water.

Promega Reagents Cleaning Procedure

One of the Promega Dual Luciferase Assay reagents, the Stop & Glo^{TM} Reagent, has a slight affinity to plastic materials. To avoid cross contamination when changing reagents, we recommend following the cleaning procedure outlined below.

To retain the fluids in the syringes for approximately 30 minutes, open the priming dialog and act according to the description in chapter 4.4. Select the maximum total priming volume recommended for your sample format.



Place a new sample tube into the instrument for each cycle! Proceed as follows:

- 1. Wash with distilled water
- □ Proceed as described above.
- 2. First cleaning step
- Fill the respective injector with **Ethanol (70%)**.
- □ Leave this solution in the system for approximately 30 minutes.
- □ Rinse injector system with distilled water.
- 3. Rinse injector system again with distilled water.

11.6 Decontamination of Injector System

If the outer surfaces of the injection system have been contaminated with potential biohazard material or with other kinds of pollution, they can be treated according to the description for decontamination of surfaces in chapter 11.1.

If it is necessary to decontaminate the pumps, tubing and injector head, proceed in the following way:

- □ Clean the respective injector system with the Acid/Base cleaning procedure.
- Fill the respective injector with **70% Ethanol** or **Isopropanol**.
- □ Leave this solution in the system for approximately 10 minutes.
- **□** Rinse injector system twice with distilled water.

Place a new sample tube into the instrument for each cycle!

The injector system has to be decontaminated before storage and shipment and in cases of contamination. It is recommended to decontaminate the injector system at least once a year.



No fluid should ever enter the instrument! If this happens, disconnect the device from mains and call for service!



11.7 Installing Tubing Connections

If a tubing connection (at the pump head or injector head) is faulty, or if you want to replace an injector tubing, you have to provide a new tubing connection. An Injection Tubing Set is supplied with the instrument. Please refer to the enclosed description.

11.7.1 Connections at the Injector Pumps

□ Drain pump: insert sample tube and run a wash cycle with several shots without having a bottle of liquid connected.



- □ Turn **Sirius L** off and disconnect the power cable from mains.
- □ Unscrew the old tubing's fitting screw at the pump head and put tubing aside.
- The Injection Tubing Set provides converted injection tubing for the injection line (black) and for the sucking line (white).
 For black and white tubing: The tubing end with the white fitting screw must be used for connection to the pump head.
- Insert new tubing with T-piece into the threaded bore hole of the pump head, turn the white fitting crew with its washer into the threading of the pump head and finger-tighten it. Do not use any tools.

Figure 11-1: Tubing connections at the pump head



Black tubing from pump to injector head

White tubing from bottle to pump









The pump thread is made of soft Teflon material. Make sure the screw does not get jammed in the screw thread.

11.7.2



Connection at Injector Head

Please take extreme care when replacing screw fittings at the injector head, as the measurement chamber opens directly above the detector. Although the sensitive detector system is protected against incident light by a shutter, which is automatically closed when the luminometer is switched off, do not leave the instrument open for a longer time. Always work as quickly as possible when removing the tubing connections.



Please follow the installation steps described below to rule out damage to the detector!

- Drain injector system: insert sample tube and run a wash cycle with several shots without having a bottle of liquid connected.
- **U** Turn **Sirius L** off and disconnect the power cable from mains.
- □ Unscrew tubing connections from the pump.
- □ To open the connection at the injector head push back the protective rubber of the tubing, untie the black fitting screw at the injector head and put black tubing aside. For exchange of a tubing connection replace only the tubing with the black fitting screw. If servicing the injector tip is not required, do not untie or replace the Banjo nut of the injector tip assembly.





- □ The Injection Tubing Set provides converted black injection tubing for the injection line. Insert the black fitting crew at the tubing's end with its T-piece into the Banjo nut of the respective injector tip assembly. Turn fitting screw into the threading and finger-tighten it. Don't use any tools!
- □ Then reconnect the tubing to the pumps again. Refer to chapter 11.7.1 for details.



11.8 Exchange Injector Tips



Please take extreme care when replacing screw fittings at the injector head, as the measurement chamber opens directly above the detector. Although the sensitive detector system is protected against incident light by a shutter, which is automatically closed when the luminometer is switched off, do not leave the instrument open for a longer time. Always work as quick as possible when removing the injector tips or parts of the injector tip assembly.



Please follow the installation steps described below to rule out damage to the detector!

- Drain injector system: insert sample tube and run a wash cycle with several shots without having a bottle of liquid connected.
- **U** Turn Sirius L off and disconnect the power cable!
- □ Unscrew tubing connections from the pump.

11.8.1 Configuration of the Injector Tip Assembly

The injector tip assembly consists of three parts:

- □ Injector tip, located inside the injector head
- black Banjo nut
- □ Teflon insert, located inside the Banjo nut

The Teflon insert must be pushed into the Banjo nut up to the tapering point. It seals the tubing system towards the tip. The injector tip is located inside the injector head. These parts are fitted into each other, when the Banjo nut with Teflon insert is screwed into the injection head with injector tip inside.







11.8.2 Replacing Injector Tips

The injector tips are located inside the injector head.

- Description Push the protective rubber up at its thicker end.
- □ Unscrew the black Banjo nut with the Teflon insert from the injector head. **Do not unscrew the injector head!**
- □ Use the widening tool to unscrew and remove the old injector tip, located inside the injector head..
- □ Insert a new injector tip into the injector head.
- □ Screw Banjo nut with Teflon insert into the injector head (finger-tight do not use any tools!).
- □ Screw the black fitting screw of the black tubing into the Banjo nut and cover it with the protective rubber.





11.8.3 Replacing the Pump Syringe

Faulty (e.g. leaky) pump syringe must be replaced.

Figure 11-6: Design of a Cavro pump



- □ Drain pump by running a wash cycle with several strokes without liquid.
- **U** Turn Sirius L off and disconnect power cable.
- □ Open knurled screw at the syringe. Detach it from the pump head, and push it down with the syringe piston holder.
- □ Take the syringe from the front out of the syringe piston holder.
- □ Insert new syringe in the syringe piston holder, push it up and fix it with the knurled screw to the pump head.
- □ Reconnect power and turn instrument back on.



12. Troubleshooting

In cases of malfunction or not expected measurement values refer to the troubleshooting list and/or contact your local distributor or Berthold Detection Systems.

Detected fail	Possible reason	Reaction
Background value >100 RLU/s	Pollution of sample holder and measurement chamber with luminescent material	Clean sample holder and measurement chamber. Refer to chapter 3.3 and 11.
	Pollution with luminescent material inside the instrument	Service required; contact BDS
	Blind screw, injector head or fitting screw of injector tubing on top of the instrument not tightened	Check blind screw or all connections to be tightened; refer to chapter 2 for details
	Instrument has been set up in a suboptimal environment	Check installation procedure for compliance with operating conditions
	In cases of a blind screw on top of a non injector instrument: blind screw has been touched during measurement	Repeat measurement without touch; contact BDS in cases of further fail



	Operation conditions outside specified range	Operate instrument only within the specified humidity and temperature range; refer to the Technical data
	Sample tubes exposed to bright light or handled immoderate	Protect tubes from bright light and minimize handling
Background or measurement value to low (0 RLU/s)	Detector or electronics damaged	Service required; contact BDS
	Instrument switched off or not connected to mains	Check connection to mains and switch on the instrument
Unexpected or wrong measurement value	Sample holder not readily fixed→ Sample position suboptimal	Check the screws of the sample holder to be tightened: chapter 2.
	Injectors are not primed correctly	Refer to chapter 4.4.
	Injector lines contaminated or blocked	Clean the injector system . Refer to chapter 11.
	Wrong measurement protocol used	Check the protocol settings.
Measurement value to high	Pollution of sample holder and measurement chamber with luminescent material	Clean sample holder and measurement chamber. Refer to chapter 11.

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	Pollution with luminescent material inside the instrument	Service required; contact BDS
	Pollution of the injector system with luminescent material or other kinds of troublesome solutions	Clean and decontaminate injector system. Refer to chapter 11.5.
	Injector head, fitting screw of injector tubing or blind screw on top of the instrument not tightened or removed	Check all connections to be tightened; refer to chapter 2 for details. Do not service an instrument when it is connected to mains and switched on !
Unexpected Overload signal of 30e6 RLU/s	Too much light	Dilute the sample
	Shutter fail	Service required; contact BDS
Sample does not really fit the sample holder (too big, too small, jiggling)	Wrong sample format for the desired sample holder	Refer to chapter 2 and select a sample format specified for your sample holder
Injector not primed	Injector not enabled	Enable the respective injector in the priming dialog
Lines and pumps empty after priming	Lines are not connected to the respective reagent supply bottle	Connect the lines to the reagent supply bottles

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Lines not totally filled after priming	Total volume smaller than the dead volume of tubing	Prime again or select a higher total volume
Overflow during priming	Total priming volume too big for the selected sample tube	Clean sample holder and measurement chamber; select a smaller total volume adequate to your sample tube → refer to chapter 4.4
		If fluids entered the instrument, disconnect the it from mains and call the service
Injector does not work	Injector fail	Service required; contact BDS
	Injector has not been selected in the PC protocol	Check your PC protocol and activate the respective injector
Injector works, but does not inject reagent	Reagent bottle connected to the wrong injector line	Always check the reagent supply bottles to be connected to the right injector number and line.
	Injector lines are not dipped into the reagents	Check the lines to be dipped into the reagents
Wrong injector used	Injectors confounded	Always check the reagent supply bottles to be connected to the right injector number and line.

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	Wrong injector selected at the measurement software	Select the right injector in the measurement protocol
Wrong injection volume	Wrong volume selected	Adjust injection volume
	Lines blocked	Refer to chapter Maintenance for Cleaning procedures
Overflow during injection	Injection volume to big	Check the total reaction volume to be within the range for your sample tube; refer to chapter and adjust your assay system
		If fluids entered the instrument, disconnect it from mains and call the service
Injector tips leaking	Old injector tips	Maintain the injector tip assembly.
Injector tubing leaking	Screws of the injector system not tightened	Finger-tighten white/black fitting screw



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13. Technical Data

13.1 Luminometer

Sample format	Tubes up to 12 mm diameter and 75 mm length, microfuge tubes 1.5 ml and 2 ml, 35 mm culture dishes, liquid scintillation vials up to 20 ml.
	Injection operation with tubes 12 mm x 75 or 55 mm and microfuge tubes 1.5 and 2 ml
Detector	Photomultiplier tube with bialkali cathode, effective spectral range 300-600 nm, operated in photon counting mode
Measurement Chamber	Retractable drawer with interchangeable reflectors and sample adapter
Sensitivity	Less than 0,5 zmol firefly luciferase, 1 amol ATP in a HS ATP assay
Dynamic range	6 decades
Interfaces	RS 232 or USB interface for PC connection
Injector	0, 1, 2 (upgrade possible)
Injection volume	20 – 500 μl adjustable in steps of 1 μl
Tubing	Chemically inert PTFE tubing and connections (PTFE or KEL-F)
Dimensions	W 349 mm, D 150 mm, H 241 mm (without injection tubing) W 349 mm, D 185 mm, H 367 mm (with injectors and lines)
Weight	approx. 3,6 kg (5.4 kg with 2 injectors)
Power supply	Desktop power supply and power cord 100-240V AC 24V / 2,5A DC
Temperature range and humidity	Storage 0° - 40° C up to 80% humidity (@30°C), non condensing Operation 10° – 35°C up to 70% humidity
Transport conditions	-25° to +60°C, up to 75% humidity, in original cardboard box and free of liquids.

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13.2 **PC Software**

PC software	MS Windows [®] application for control, measurement and evaluation
Platform/ Required Hardware	Microsoft Windows compatible PC, Pentium like processor, RS 232 or USB port
Operating System	Windows [®] 2000, XP, Vista, Windows [®] 7; 32 bit
Additional Software	Microsoft Excel [™] 2000 or higher (optional)
Standard configuration	Protocol Manager, Quick Measurement
Additionally available	Single Assay, Dual Assay, Single Kinetics, Multiple Kinetics, Cut- off Assay

Accessories 13.3

Order number	Description
18100026	Reflector for microfuge tubes
13200500	5 ml tubes, 12x75 mm, 500 each
14000130	Luminescent TestTube for validation of Tube Luminometers

For spare parts please contact your local distributor

or service@titertek-berthold.com



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14. **Preparing Sirius L for Transport**

If it should become necessary that Sirius L has to be serviced please observe the following instructions for shipping it to your local distributor or Berthold Detection Systems.

- 1. Turn luminometer off and disconnect power supply.
- 2. Clean the instrument according to the instructions for cleaning and decontamination listed in the decontamination form.
- 3. For safe shipment put the **luminometer and the accessory box** into the original cardboard box and seal it. If the original box is not available please contact <u>service@titertek-</u> <u>berthold.com</u>. We will send a box back to you.



4. Before return please contact your local distributor or Berthold Detection systems for shipping instructions.



15. Decontamination Form

Any laboratory instrument used for clinical or research analysis maybe considered a biohazard and requires decontamination prior to handling. Universal precautions are suggested wherever applicable.

Before shipping any instrument back to Berthold Detection Systems the luminometers have to be cleaned in the following way:

- 1. Wash the housing with a moist cloth, if necessary use a mild detergent or 70% Isopropanol.
- 2. Use 70% Isopropanol for sterilizing the injection lines after 10 minutes incubation time. Rinse with distilled water thoroughly.
- 3. Empty the injection lines.
- 4. Open the access door, remove and clean the reflector, then clean the measurement chamber with the measurement window carefully. Decontaminate the instruments surfaces, measurement chamber and sample holder with 70% Isopropanol according to the description in the chapter "Maintenance". Install the reflector again according to the description.
- 5. Use cotton tipped applicators for hard to reach areas, if necessary.

Berthold Detection Systems only accepts instruments for repair together with a filled out decontamination form. Thank you.

Instrument Type:_____

Serial Number:_____

I confirm that the specified instrument was decontaminated according to the above described decontamination procedure

I confirm that the above specified instrument had no contact to any hazardous material.

Company	
Contact Person	
Position	
Telephone	

Date:_____ Signature: _____

Please copy and fax to:

Berthold Detection Systems, Bleichstr. 56-68, 75173 Pforzheim, Tel.:+49-(0)7231-9206-0, Fax –50



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