

CLARIOstar[®]

Operating Manual

Revision B

CLARIOstar®

The CLARIOstar is a multifunctional microplate reader capable of performing a wide array of applications for fluorescence intensity, fluorescence polarization, time-resolved fluorescence, TR-FRET (e.g. HTRF®), luminescence, AlphaScreen®, and full spectrum absorbance. Using BMG LABTECH's proprietary technology, the CLARIOstar delivers superior results in all plate formats and in all detection modes.



CLARIOstar

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This manual was designed to guide CLARIOstar users through the basic hardware features of the microplate reader.

Although these instructions were carefully written and checked, BMG LABTECH cannot accept responsibility for problems encountered when using this manual. Suggestions for improvement will be gratefully accepted.

BMG LABTECH reserves the right to change or update this manual at any time. The revision number is stated at the bottom of every page.

For contact information please visit our website at www.bmglabtech.com or send an email to germany@bmglabtech.com.

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Table of Contents

1	Technical Specifications	5
2	Safety Information	7
2.1	Description of Warnings	7
2.2	General Information	7
2.3	Environmental Safety Standards	8
2.4	Electrical Safety	8
2.5	Photobiological Safety	8
2.6	Light and Laser Safety	9
2.7	Chemical and Biological Safety	9
2.8	Instrument Disinfection	10
2.9	Disposal and Instrument Takeback / Recycling Program	12
2.9.1	DISPOSAL OF TRANSPORT PACKING	12
2.9.2	WEEE COMPLIANCE	12
3	Overview	13
3.1	Front and Inside of CLARIOstar	13
3.2	Back of CLARIOstar	14
4	Installation	15
4.1	Transport Locks—Plate Carrier and Needle Holder	16
4.1.1	UNLOCKING THE PLATE CARRIER	16
4.1.2	UNLOCKING THE NEEDLE HOLDER	16
4.2.1	LOCKING THE PLATE CARRIER	17
4.2.2	LOCKING THE NEEDLE HOLDER	17
5	Description of Components and Peripherals	18
5.1	CLARIOstar Operating Light	18
5.2	Plate Carrier In/Out Button	18
5.3	Installing and Changing Filters	19
5.4	Descriptions of LVF Monochromators™ and Filters	21
5.4.1	LVF MONOCHROMATOR™	21
5.4.2	FILTERS	21
5.5	Reagent Injection System	22
5.5.1	REAGENT INJECTORS	22
5.5.2	ACCESSING REAGENTS	23
5.5.3	NEEDLE HOLDER	23
5.5.4	APERTURE SPOON	24
5.5.5	HOLDER FOR REAGENT COMPOUND VESSELS	25
5.5.6	PUMP PRIMING	25
5.6	Automatic Height Sensor	26
5.7	CLARIOstar - Stacker Connection	27
6	Troubleshooting	28
6.1	Cleaning the Dust Filters	28
6.2	Locking the Plate Carrier Manually	28

1 Technical Specifications

Detection Modes	<ul style="list-style-type: none"> - Fluorescence Intensity - including FRET - Luminescence (flash and glow) - including BRET - UV/Vis Absorbance - Fluorescence Polarization / Anisotropy - Time-Resolved Fluorescence - including TR-FRET - AlphaScreen® / AlphaLISA®
Measurement Modes	<ul style="list-style-type: none"> - Top and Bottom reading - Endpoint and Kinetic measurements - Spectral Scanning (Fluorescence, Luminescence and Absorbance) - Well Scanning
Microplate Format	<ul style="list-style-type: none"> - 6- to 1536-well plates, LVis Plate with 16 microspots (2 µL)
Light Sources	<ul style="list-style-type: none"> - High-energy xenon flash lamp - Dedicated Laser for AlphaScreen® / AlphaLISA®
Detectors	<ul style="list-style-type: none"> - Low noise photomultiplier tube (PMT) - UV/Vis Absorbance spectrometer
Dual LVF Monochromator™	<ul style="list-style-type: none"> - Fluorescence Top and Bottom - Luminescence Top and Bottom - Fluorescence Excitation / Emission Spectral Scanning - Luminescence Emission Spectral Scanning - Spectral Range: 320 - 850 nm (selectable increments from 0.1 to 10 nm) - Software Selectable Bandwidths: 8 - 100 nm
Linear Variable Dichroic Mirror	<ul style="list-style-type: none"> - Spectral Range: 340 - 740 nm (selectable increments of 0.1 nm)
UV/Vis Absorbance Spectrometer	<ul style="list-style-type: none"> - Spectral Scanning or up to 8 discrete wavelengths in less than 1 sec / well - Spectral Range: 220 - 1000 nm (selectable increments from 1 to 10 nm) - Bandwidth: 3 nm
Optical Filters	<ul style="list-style-type: none"> - Top and Bottom for all detection modes, except absorbance - Up to 4 excitation filters, 4 emission filters, and 3 dichroic mirrors - Spectral Range: 230 - 900 nm
Sensitivity	<ul style="list-style-type: none"> - FI Filters (top): 0.4 pM Fluorescein, 384sv, 20µL (< 8 amol/well) - FI Filters (bottom): 1.0 pM Fluorescein, 384, 50µL (<50 amol/well) - FI Monochromator (top): 0.5 pM Fluorescein, 384sv, 20µL (< 10 amol/well) - FI Monochromator (bottom): 3.0 pM Fluorescein, 384, 50µL (< 150 amol/well) - FP: < 0.7 mP SD at 1 nM Fluorescein, 384sv, 20µL - TRF: < 40 fM Europium, 384sv, 20 µL (< 0.8 amol/well) - HTRF: HTRF® certified for black and white microplates Reader Control Kit (Eu) after 18h incubation, 384sv, 20 mL > 880 % Delta F for High Calibrator > 30 % Delta F for Low Calibrator < 2.0 % CV for Standard 0 - LUM: < 0.4 pM ATP, 384sv, 20 µL (< 8 amol/well) Dynamic Range: 7 decades - AlphaScreen®: < 100 amol P-Tyr-100 (384sv, 20µL) - ABS with Spectrometer Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD Dynamic Range: 0 - 4 OD

Read Times	- 1 flash: 8s (96), 15s (384), 28s (1536)
Reagent Injectors	<ul style="list-style-type: none"> - Up to two built-in reagent injectors - Injection into 6-well up to 384-well plates - Individual injection volumes for each well (3 to 350 μL) - Variable injection speed (up to 420 μL/s with a 500 μL syringe) - Up to four injection events per well - Reagent back flushing
Gain Control	- Software selectable gain for F, TRF, FP, L, and AS.
Height adjustment	- Automatic focal height adjustment (0.1 mm resolution) with curve monitoring
Temperature Control	<ul style="list-style-type: none"> - Incubation range from 3°C above ambient to 45°C or 65°C - Temperature monitoring (with and without incubation)
Plate Carrier	<ul style="list-style-type: none"> - Auto lock microplate carrier - All microplate formats up to 1536-well in all detection modes - Microplates should adhere to the SBS specification and non-SBS formats should fit: (LxWxH) (mm) max: 128x86x20; min: length 124
Shaking	<ul style="list-style-type: none"> - Double orbital, orbital and linear shaking - Programmable shake time and speed
Computer interface	- USB 3.0, USB 2.0, cable with USB 2.0 AB connectors
Power requirements	<ul style="list-style-type: none"> - 100 or 115 or 230 V, 50/60 Hz, auto ranging - Consumption: Normal operating conditions: 50 W <li style="padding-left: 20px;">Idle State: 20W <li style="padding-left: 20px;">Switched off: 0W <li style="padding-left: 20px;">max. 300 VA
Fuses	- T 5A/250V for main power 100 - 240 V (use original type Wickmann only)
Dimensions and weight	- Height: 40 cm, width: 45 cm, depth: 51 cm, weight: 32 kg max.
Ambient conditions	<ul style="list-style-type: none"> - Operating temperature: 15°C to 35°C - Storage temperature: -10°C to 50°C - Humidity of atmosphere: 20 % to 80 % - Non-condensing environment.
Instrument conformity	- Over voltage category II; pollution degree II; protection class I; Laser class I
Robotic capabilities	<ul style="list-style-type: none"> - Easy to integrate into all existing robotic systems <p>For medium level throughput, an optional BMG LABTECH Stacker with magazines for 50 microplates is available</p>

The fluorescence ratio associated with the HTRF[®] readout is a correction method developed by Cisbio international and covered by the US patent 5,527,684 and its foreign equivalents, for which Cisbio international has granted a license to BMG LABTECH. Its application is strictly limited to the use of HTRF[®] reagents and technology, excluding any other TR-FRET technologies.

* Limit of detection < 100 amol of biotinylated and phosphorylated polypeptide (P-Tyr-100 assay kit, PerkinElmer, #6760620C), measured in white 384 small volume microplates (17 μ L/well).

AlphaScreen, DELFIA, and LANCE are registered trademarks of Perkin Elmer, Inc.

HTRF is a registered trademark of Cisbio international.

LanthaScreen is a registered trademark of Invitrogen Corp.

Transcreener is a registered trademark of BellBrook Labs

Specifications are subject to change without notice.

2 Safety Information

2.1 Description of Warnings



A general warning calls attention to a circumstance which is described below. User must follow corresponding instructions strictly.



Warning for optical radiation



Warning for laser beam

2.2 General Information



This instrument must be installed and used as outlined in this Operating Manual. Installation, service, and any operation which requires opening of the instrument must be performed only by trained and certified personnel from BMG LABTECH. Failure to comply with these instructions will impair or even invalidate the warranty and can lead to unsafe operation of this equipment.



The area designated for the instrument should be free of dust, liquids, and acidic vapor. The surface of the table should be flat and even. Avoid areas subject to vibrations and direct sunlight.



Prior to turning on the instrument for the first time, let the instrument adapt to room temperature for at least 3 hours to avoid condensation which can cause a short circuit. BMG LABTECH will void the warranty if damage occurs to electrical and/or mechanical parts in cases where the instrument was turned on before the recommended accommodation time.



Handling and operation of the equipment must be carried out only by qualified personnel and staff trained by an official BMG LABTECH representative.



Microplates and related accessories (e.g. LVis Plate) operated at temperatures higher than 55°C must cool down below 55°C before being handled and removed from the instrument.



Samples and reagents in solid, liquid, or gaseous form must be removed from the instrument immediately after measurement to avoid corrosion and accumulation of hazardous substances inside the instrument.

2.3 Environmental Safety Standards

The environmental safety standards for operation under norm IEC 61010-1 are met under the following conditions:

- Indoor use (adhere to the Occupational Exposure Limit Values for ECM, UPS, vibration, and sunlight when setting up the instrument in the laboratory)
- Altitude (up to 2000 m)
- Temperature (+15°C to +35°C)
- Relative Humidity (Maximum 80% at 31°C non condensing then decreasing linearly to 50% at 40°C)
- Mains supply voltage fluctuation (+/- 10%)
- Overvoltage category (II) acc. to IEC 60364-4-443
- Pollution degree (2) acc. to IEC 61010-1

2.4 Electrical Safety

- Connect the unit only to a grounded supply socket. The instrument is class 1 construction and must be grounded.
- Connect the unit only to a power supply with a designated voltage rating corresponding to the label on the back of the instrument.

2.5 Photobiological Safety

This instrument is rated in the free group as RG 0, according to the IEC 62471-2 photobiological safety of lamps and lamp systems. The intended and routine use of this equipment and its high power xenon flash lamp does not pose a photobiological hazard, given that the user strictly follows these precautions:



Do not operate the instrument unless the instrument shell is mounted and all screws are in place and tightened.



Do not open the flap door of the microplate carrier during a measurement run.



Do not open the lid during a measurement run.

A measurement run is indicated by a blinking purple LED on the instrument.

2.6 Light and Laser Safety

This instrument is rated as a class 1 laser product, according to the IEC 60825-1 safety of laser products. The intended and routine use of this equipment and its laser light source does not pose a health risk, given that the user strictly follows these precautions:



Do not operate the instrument unless the instrument shell is mounted and all screws are in place and tightened.



Do not open the flap door of the microplate carrier during a measurement run.



Do not open the lid during a measurement run.

A measurement run is indicated by a blinking purple LED on the instrument.

The following light sources are incorporated and encapsulated in the CLARIOstar microplate reader. The Xenon flash lamp is standard, and the solid state AlphaScreen laser is optional.



Xenon high power flash lamp – emits ultraviolet light during operation at levels that could injure the eyes or skin. Thus avoid looking at the light directly and avoid exposing any skin to the light. During normal operation the user will not encounter any light from the xenon flash lamp.



Solid state AlphaScreen[®] laser – laser class 3B. Emits light pulses at $\lambda=680$ nm with <15 mW that could injure the eye. Thus avoid looking at the laser light directly. The laser is embedded inside the instrument and during normal operation the user will not encounter any light from the AlphaScreen[®] laser. The instrument is rated a class 1 laser product.

2.7 Chemical and Biological Safety

Daily routine use with this instrument may involve the handling and use of compounds that are toxic, flammable, or biologically harmful. When working with materials and compounds as stated, make sure to observe the following precautions:

- Handle all samples in liquid, solid, or in gaseous form according to good laboratory practice.
- Adhere to the maximum workplace concentration (MAC) and to laboratory safety regulations (e.g. BGI 850-0, formerly BGR120 in Germany).
- Wear safety goggles since spilling of liquids may occur.
- Contact your safety officer to dispose of hazardous waste solutions and when working with flammable liquids.

The CLARIOstar must not be exposed for any length of time to chlorinated hydrocarbons at high concentration (i.e., chloroform, aromatic hydrocarbons, such as toluene or benzene, etc.) or to acetone. All warranties are void if the instrument comes in contact with organic solvents.

If using any of these substances while performing a measurement, be sure to remove the sample immediately after measurement is completed. If evaporation of any of these substances occurs inside the instrument, it is important to vent the instrument for a sufficient amount of time by opening the lid and placing the microplate carrier in the plate out position with the flap door open.



Use only mild detergent or 70% ethanol for cleaning the instrument. Make sure the instrument is always in the OFF position for cleaning and servicing.

2.8 Instrument Disinfection

Please follow all instructions carefully for a successful disinfection of this instrument.

All parts of the instrument, which have the possibility of contacting patient sera or positive samples, have to be handled as if they are hazardous. For this reason, it is recommended that gloves be worn while maintaining or working with the instrument.

It is very important that the instrument is thoroughly disinfected before maintenance or before removing the instrument from the laboratory. Be sure that the instrument is disinfected before you send it to your distributor or to the manufacturer. For safety reasons, you have to fill out the Disinfection Certificate, or the instrument may not be accepted by the service center or by customs authorities.

Use suitable disinfectants, e.g. alcohol (70%).

Authorized personnel wearing disposable gloves and protective clothing should only perform the disinfection procedure. The location should be well ventilated.

Disinfection Steps

1. Disconnect the instrument from the main power supply.
2. Remove the USB cable from the connector.
3. Clean all outside surfaces of the instrument carefully with cotton wool, which has been soaked in disinfecting solution.
4. Place the instrument in a large plastic bag along with the cotton wool that has been soaked in disinfecting solution. Ensure that the wool does not touch the instrument.
5. Close and seal the bag.
6. Keep the instrument in the plastic bag for at least 24 hours.
7. After the disinfection time has lapsed, remove the instrument from the plastic bag and clean all outside surfaces of the instrument with cotton wool that has been soaked in alcohol solution.
8. Repeat the procedure for disinfection on any accessories, which will be returned with the instrument.
9. Complete the Disinfection Certificate.

Disinfection Certification

This instrument and its inventory have never been in contact with any dangerous biological material, or if so, the instrument and its inventory have been disinfected according to the instructions given in the Operating Manual.

Name: _____

Firm: _____

Date, Signature: _____

2.9 Disposal and Instrument Takeback / Recycling Program

We strongly recommend keeping the instrument shipping box in case you need to send the instrument back for upgrade or repair. When disposing of the unit, please adhere to the following guidelines:

2.9.1 Disposal of Transport Packing

Straps to fix the packing on pallet, edge protection, air cushion styrofoam, and plastic foil must be disposed of and recycled according to your local "plastic recycling" policy.

Transport cardboard box must be disposed of and recycled according to your local "paper recycling" policy.

2.9.2 WEEE Compliance

The European Union Waste Electrical and Electronic Equipment Directive (WEEE) is a Producer Responsibility Directive aimed at reducing the waste from electrical equipment, increasing recovery and recycling rates of WEEE and improving environmental performance of all operators involved in the life cycle of electrical and electronic equipment.

The directive specifies that, as of August 13, 2005, manufacturers of specified electronic equipment will be obliged to take back these products at the end of their useful life.

BMG LABTECH will comply with the WEEE take back requirements for BMG LABTECH branded product sold in the EU after August 13, 2005.

BMG LABTECH customers based in Europe can take advantage of the BMG LABTECH Takeback and Recycle Program. Please check BMG LABTECH's website for country-specific availability.

This Takeback and Recycle Program ensures that you can ship the product back to BMG LABTECH for proper handling at the end of its useful life. Disposal will be carried out in an environmentally safe manner using processes that comply with all current eWaste regulations where applicable.

Upon completion of the recycling process BMG LABTECH will provide, on request, a Certificate of Destruction (COD). This will release your business from any further liability for the equipment.

The program is accessible via the web at
www.bmglabtech.com/support/countryselect.cfm?page=support.cfm

3 Overview

3.1 Front and Inside of CLARIOstar

Lid to access internal components (Filters, Reagent Injection System, Transport Lock)

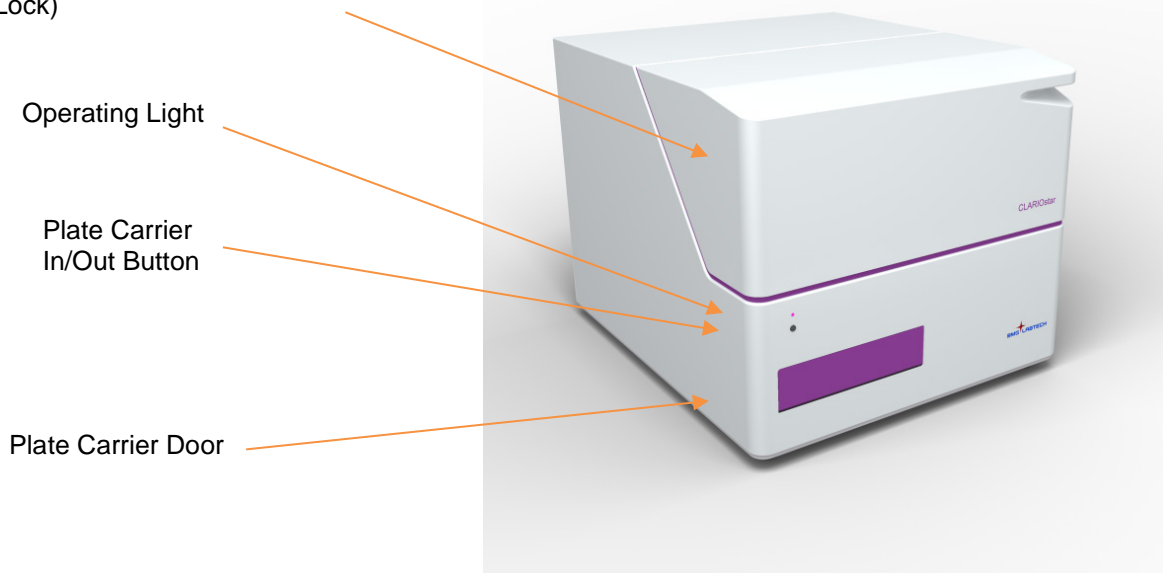


Figure 1: CLARIOstar, external view

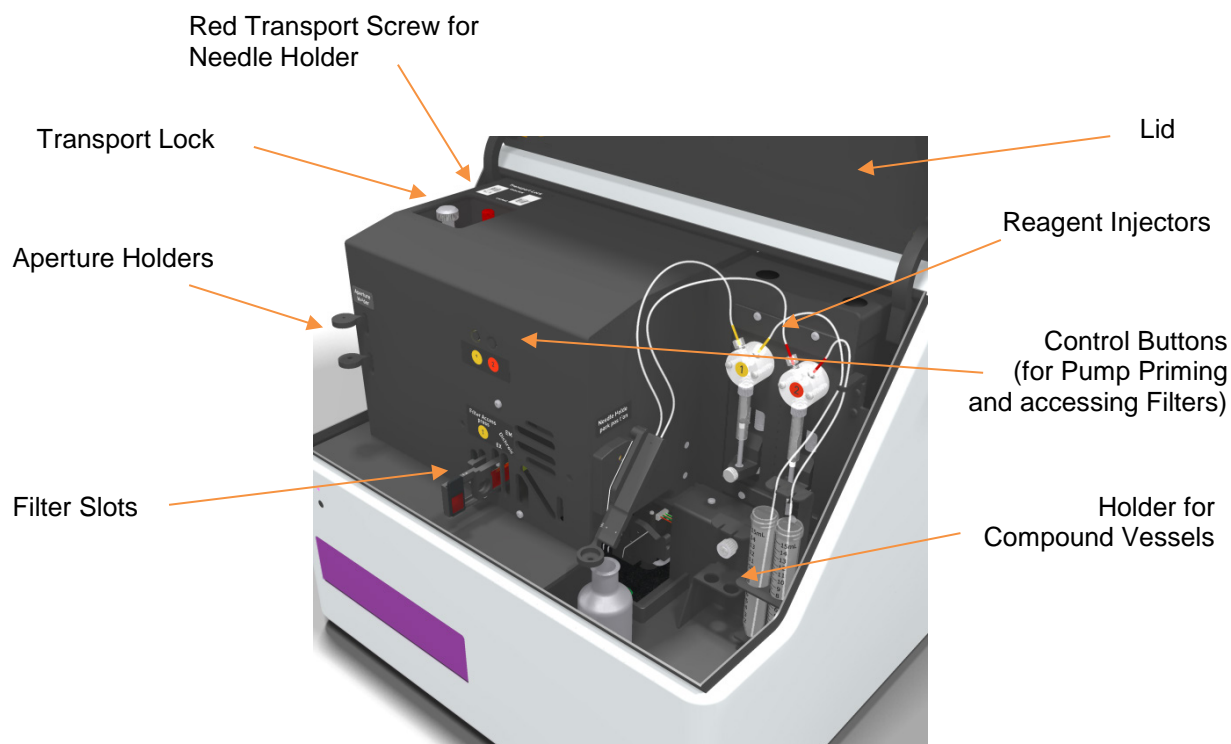


Figure 2: Internal view of the CLARIOstar under Lid, showing Aperture Holders, Filter Slots, Reagent Injection System, and Transport Lock

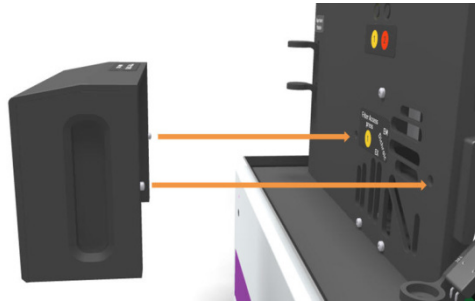


Figure 3: CLARIOstar magnetized Filter Cover

3.2 Back of CLARIOstar

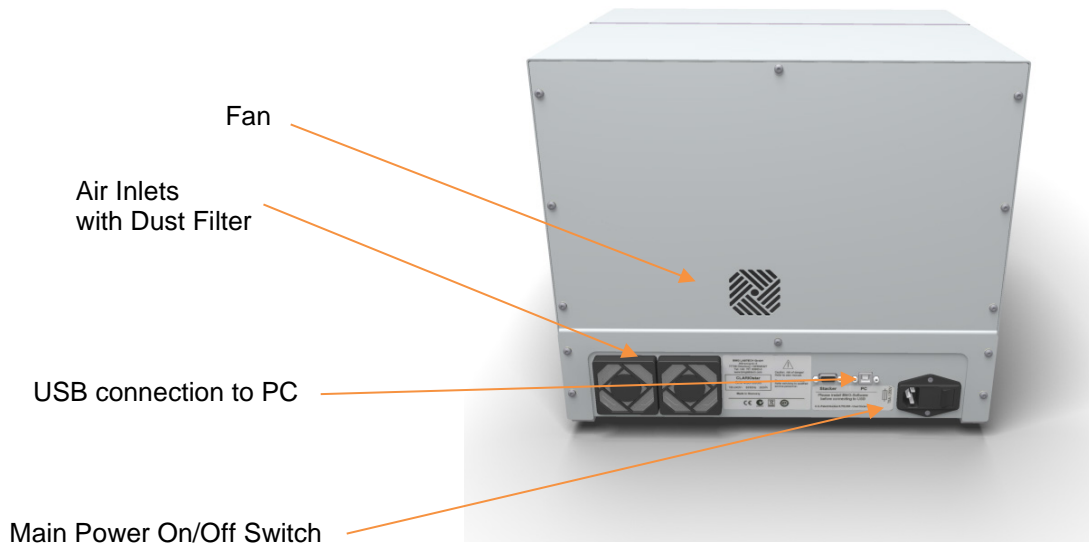


Figure 4: Back of CLARIOstar

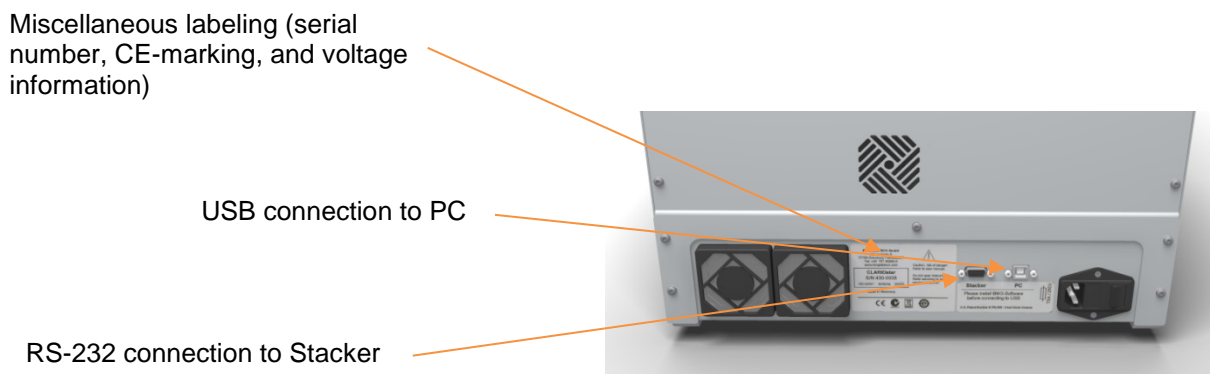


Figure 5: Back of CLARIOstar

4 Installation

Please follow these important steps to ensure successful installation of the CLARIOstar. No measurements can be performed until the instrument has been successfully installed according to these instructions.

When unpacking the instrument, please make sure that all parts are included.

The shipping box should contain:

- **CLARIOstar Instrument**
- Manual
- Control and evaluation software (CD ROM in a cover inside the manual)
- Power cord
- USB cable
- 2 extra fuses-Wickmann original: T 5A/250V for main power 100 - 240 V

Note: Other items may be included depending on instrument configuration and specific customer order.

Call BMG LABTECH immediately if any of these items are missing

The instrument should be placed in an area free of dust, liquids, and acidic vapours and on a surface that is flat and even. Avoid surfaces and areas subject to vibrations and direct sunlight. A minimum 10 centimeters of open space is required behind the instrument. Space above the instrument is required to accommodate opening of the lid.



The operator of the CLARIOstar microplate reader is assumed to be trained in the correct operation of the instrument and the safety issues.

Be careful of the CLARIOstar lid. Take care not to bump it while it is in the open position or to catch your fingers or hands beneath it.

Upon unpacking and positioning the reader, make sure to unlock the transport locks (see section 4.1.1 and 4.1.2) before the instrument is connected to the power supply.

When moving the CLARIOstar, it is recommended that two people lift the instrument together to avoid personal injury.

Lift the instrument from the bottom and both sides, as shown in Figure 5. Do not lift the instrument from the top or lid as the lid could come open.

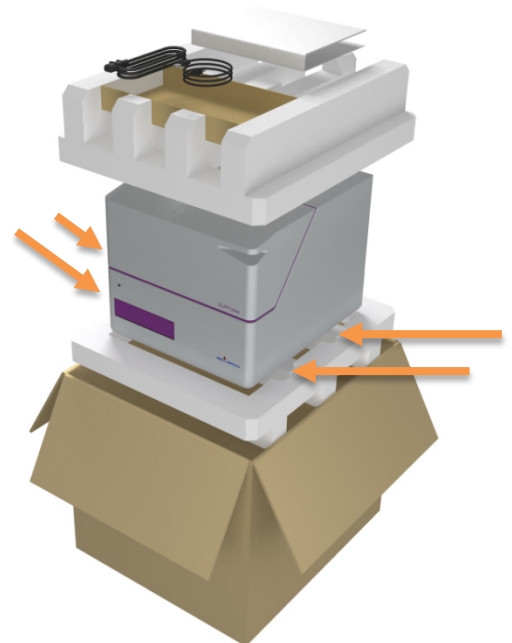


Figure 6: Moving and Packing the CLARIOstar

4.1 Transport Locks—Plate Carrier and Needle Holder

The transport locks for the plate carrier and the needle holder must be unlocked upon receiving the CLARIOstar and removing it from the shipment box.

4.1.1 Unlocking the Plate Carrier

The transport pin that locks the plate carrier can be accessed inside the instrument. Open the lid of the CLARIOstar and locate a silver screw in the middle left for locking/unlocking the plate carrier. By hand, turn the screw counter-clockwise to unlock the pin of the plate carrier (Figure 7).

If the screw moves freely up and down, then the plate carrier is unlocked and ready to be used. Once the CLARIOstar has been turned on, press the in/out button (Figure 11) briefly and the plate carrier should move out.



Figure 7: Unlocking the Transport Pin

4.1.2 Unlocking the Needle Holder

The red transport screw that locks the needle holder can be accessed inside the instrument. Open the lid of the CLARIOstar and locate the red screw on the right side of the instrument for unlocking the needle holder. By hand, turn the screw counter-clockwise to unlock (Figure 8). Remove the red screw. The needle holder is now able to be removed from its park position. Note that there is a holding place for the red screw along with the silver transport pin in the transport lock area on the top left of the instrument (see Figure 2).

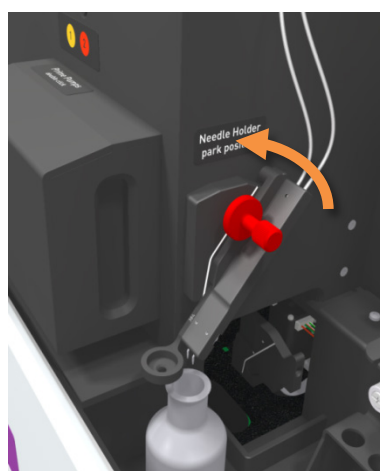


Figure 8: Unlocking the Needle Holder

4.2.1 Locking the Plate Carrier

To move the plate carrier to the locked position, close the software or push and hold the plate carrier in/out button for more than three seconds (Figure 11). Switch off the main power. To lock the plate carrier, push the silver screw down and turn it clockwise until tightened (Figure 9). The screw must be fixed tightly when locked.



Figure 9: Locking the Plate Carrier

4.2.2 Locking the Needle Holder

To lock the needle holder into its magnetized park position, first switch off the main power. Next fit the needle holder into its magnetized park position. Push the red screw in and turn it clockwise until tightened (Figure 10). The screw must be fixed tightly when locked.

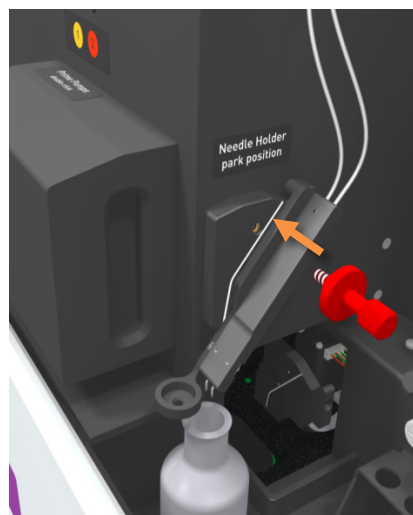


Figure 10: Locking the Needle Holder



If the CLARIOstar is moved or shipped to another location, it is very important to lock and secure plate carrier and needle holder in order to avoid damage.

5 Description of Components and Peripherals

5.1 CLARIOstar Operating Light

After switching on the CLARIOstar, the operating light will flash several times indicating the startup of the instrument's internal software. When the operating light remains constant, the instrument is in standby mode.

The operating light (see Figure 11) remains constantly purple when the instrument is turned on.

A flashing purple light (once per second) indicates the instrument is busy (e.g. performing a measurement, plate in/out, etc.).

A quickly flashing purple light (five flashes per second) indicates that the reader has stopped for some specific reason.



The function of the Plate Carrier In/Out button will be disabled during measurement and during Stacker operation!

5.2 Plate Carrier In/Out Button

The plate carrier in/out button on the front left of the instrument (Figure 11) is an easy way to move the plate carrier to the in and out positions. The plate carrier in/out button works independently from the CLARIOstar control software.

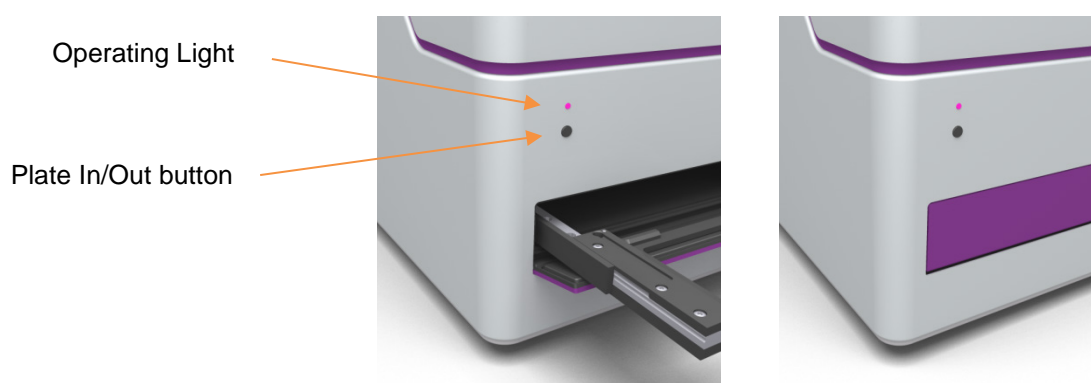


Figure 11: Plate In/Out button and Operating Light

Pressing the plate carrier in/out button for more than three seconds moves the plate carrier to the reference position in which it can be locked, as described in section 4.1.1.

5.3 Installing and Changing Filters

The CLARIOstar can accommodate up to four emission filters (Em), up to three dichroic filters, and up to four excitation (Ex) filters.



In case of shipment or moving the CLARIOstar, it is important to remove the filters from the filter slides.

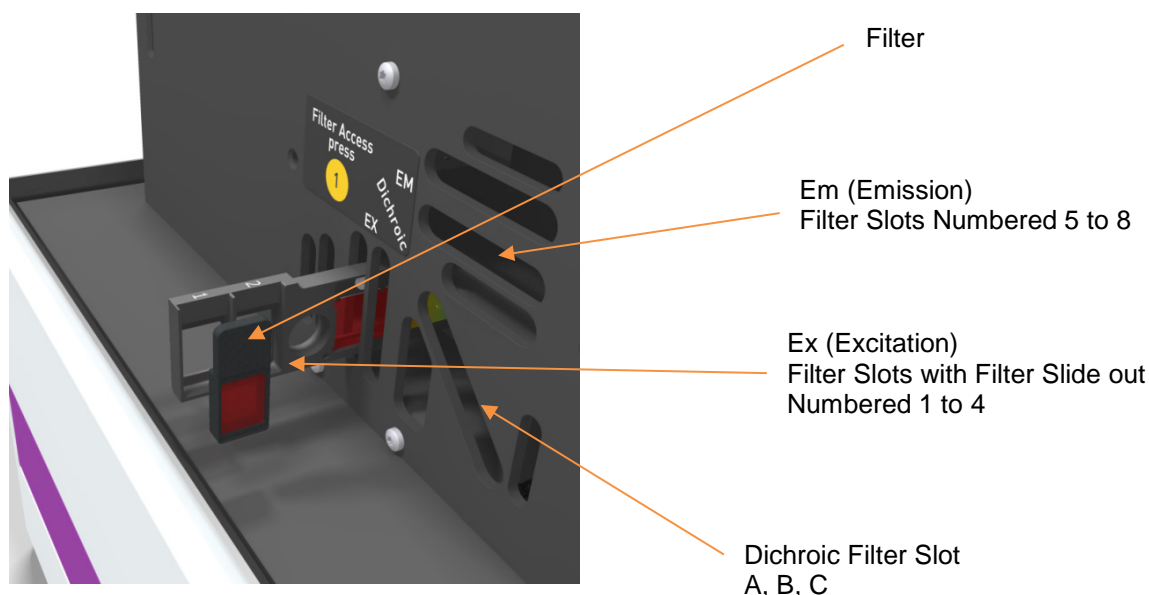


Figure 12: Internal View of CLARIOstar showing Filter Slots for Ex, Em, and Dichroic Filters, and EX Filter Slide and Filter

Filters can only be installed, changed, or removed when the CLARIOstar is powered on.

The CLARIOstar has eleven positions for filters accessible via five filter slots. Once the magnetic filter cover (see Figure 3) has been removed, filters can be installed using the filter access button (yellow button 1) to access the filter slides and move them out of the instrument and then back in. To move filter slides back into the instrument, press button 2 (labeled red).

To access filter slides, first remove the magnetic filter cover (Figure 3) to access the filter slots and slides and then push filter access button (1). Each filter slide can be accessed by repeatedly pushing filter access button (1) so that each respective filter slide emerges in succession. Place the magnetic filter into the desired filter slide and position. Filters snap magnetically into filter slides. See Figure 13 for correct placement of filters into slide.

There are two Ex (excitation) filter slides located at the bottom middle of the instrument. Ex filter slides can accommodate four filters: positions 1 and 2 in the first slide, and positions 3 and 4 in the second.

There is one dichroic filter slide located to the right of the Em filter slots and on a diagonal. The dichroic filter slide can accommodate three filters, in positions A, B, and C. (It is important to note that the dichroic filters are larger than the EM or EX filters.)

There are two Em (emission) filter slides located above the dichroic Filter slots. Em filter slides can accommodate four filters: positions 5 and 6 in the first slide, and positions 7 and 8 in the second slide.

When finished installing filters, replace the magnetic filter cover over the filter slots.

Please make sure that the filters are fitted correctly into the filter slides. Filter slides and filters are magnetized, and filters snap in to filter slide as shown in Figure 13.

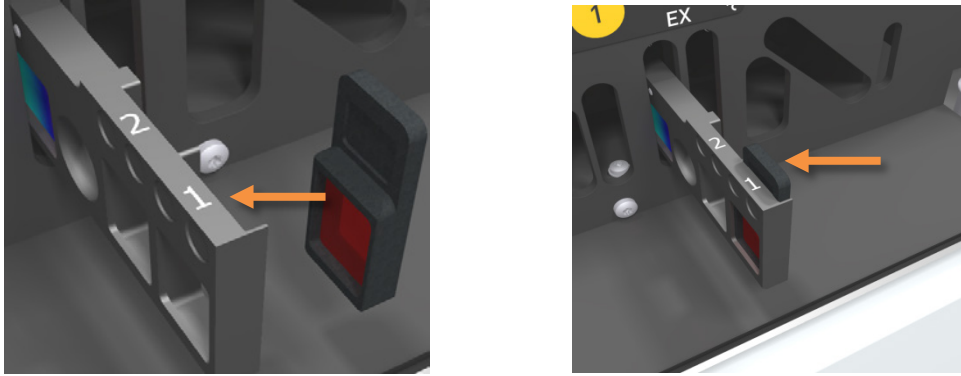


Figure 13: Correct Placement of Filters



Be careful not to touch any filter surfaces while handling the filters

The various types of filters are described in more details in Chapter 5.4.

Once software has been installed, after installing filters and before taking a measurement, it is necessary to enter the filter definitions into the filter table (Figure 14) in the control software. In the control software, the filter table is accessible by choosing 'Filter' under the 'Settings' menu. (Please see the software manual for additional information). The settings in this table are only for the user changeable filters. The monochromator settings are independent and are set in the protocols.

Filters					
Position	Excitation	Position	Dichroic	Position	Emission
1	Ex 485	A	LP 505	5	Em 520
2	Ex AS	B	SP AS	6	570-100
3	Ex TR	C	LP TR	7	665-10
4	-	-	-	8	620-10

Figure 14: Filter Table in Control Software

5.4 Descriptions of LVF Monochromators™ and Filters

5.4.1 LVF Monochromator™

Description of Technology

BMG LABTECH's advanced monochromator system consists of two Linear Variable Filter (LVF) Monochromators and Filter Selectors, one for excitation and one for emission. In addition, a Linear Variable Dichroic Mirror (LVDM) slide is used to separate the excitation from the emission light. The LVF Monochromator consists of a Linear Variable Long Pass (LVLP) slide and a Linear Variable Short Pass (LVSP) slide. The LVLP forms the rising edge while the LVSP forms the falling edge of a bandpass filter. By moving the LVLP and the LVSP slides relative to each other, the system creates a filter with a distinct variable peak wavelength and bandwidth. High transmission of light and the elimination of stray light give the CLARIOstar's monochromator system filter-like performance. In addition, the slides can hold fixed filters, polarizers, and fixed dichroic mirrors for special applications like FP, TR-FRET or Alpha Technology.

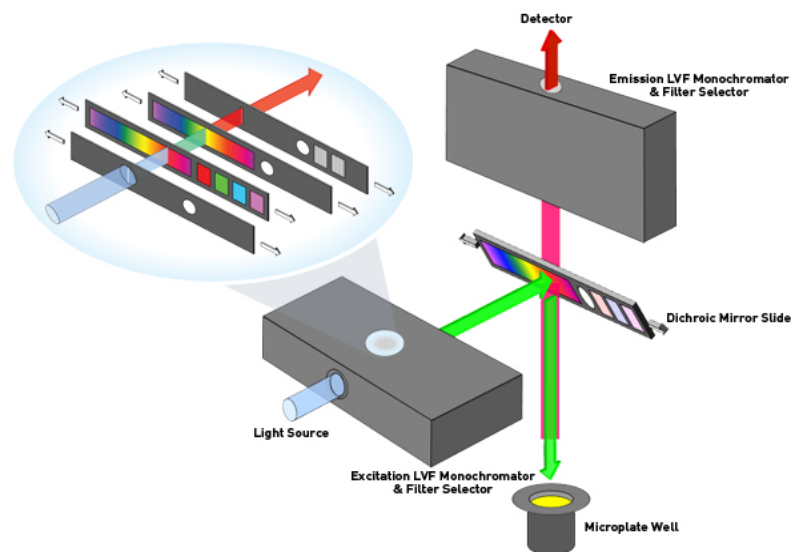


Figure 15: Overview CLARIOstar's LVF Monochromator™ System

Fluorescence Intensity and TRF

The monochromator in the CLARIOstar allows the user to choose individual filter settings.

In fluorescence intensity (FI) and Time-Resolved Fluorescence (TRF) modes, the excitation filter wavelength can be selected by using the software. The same is possible with the emission filter wavelength. The software will automatically set the correct beam splitter wavelength after setting Ex and Em filters.

Luminescence

In luminescence mode it is possible to measure the full spectrum of the sample light at once (so called standard luminescence).

In addition, a specific wavelength can be selected with the monochromator, for example, in BRET measurements.

5.4.2 Filters

To achieve the best performance possible in fluorescence polarization (FP), HTRF, AlphaScreen®, AlphaLISA®, and other more specific applications, BMG LABTECH offers dedicated filter sets, specific for each application.

FP, AlphaScreen® and AlphaLISA® filter sets consist of 1 Ex, 1 Dichroic, and 1 Em filter. Filter sets for FRET based applications, such as HTRF or Dual Chromatics like Fura-2, will include additional Ex or Em filters as required.

5.5 Reagent Injection System

The CLARIOstar can be equipped with an internal onboard reagent injection system.

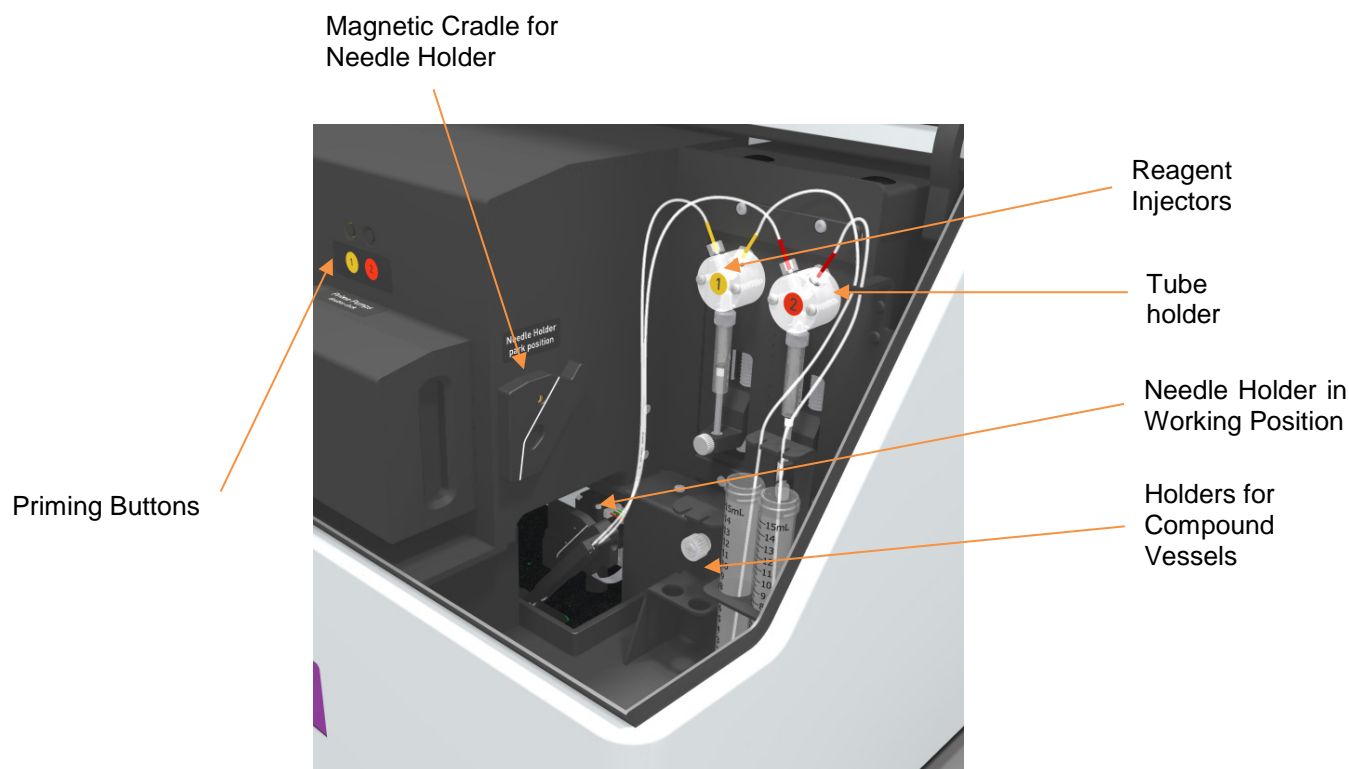


Figure 16: Overview of the CLARIOstar Reagent Injection System

The reagent needles are made of stainless steel, the tubing and valve housing are made of Teflon and Kel-F, and the syringe barrel is made of glass. All reagent injector materials are among the most chemically resistant materials that are available.

Note: Before using a pump in a test run, it is necessary to prime the pump. This can be done using the control software (menu command 'Measure | Prime'). It is also possible to prime the pumps without using the software. After opening the lid, locate the two buttons for priming. Refer to chapter 5.5.6 for a detailed description of the priming procedure.

5.5.1 Reagent Injectors

For optimal performance, it is recommended to follow these guidelines:

Do not use the syringes more than two cycles without liquid.

Flush the syringes thoroughly with distilled water after each use.

Wipe the plunger with ethanol after removing it from the syringe barrel and before replacing it.

Syringes should be cleaned weekly using one of the following procedures:

To remove cellular debris and viscous solutions from the syringe barrel:
Take off the syringe barrel and rinse it with distilled water..

Cleaning with weak detergent or 10% bleach

1. Fill the syringe with a weak detergent or 10% bleach solution.
2. Leave the solution in the syringe for 30 minutes.
3. Flush the syringe a minimum of 10 times with distilled water.

Cleaning with acid / base (Best procedure if cells are used in the syringe)

1. Fill the syringe with 0.1M NaOH and leave it in the syringe for 10 minutes.
2. Flush the syringe a minimum of 10 times with distilled water.
3. Fill the syringe with 0.1M HCl, and leave it in the syringe for 10 minutes.
4. Flush the syringe a minimum of 10 times with distilled water.

5.5.2 Accessing Reagents

Open the CLARIOstar by lifting the finger handle on the right front corner of the instrument. The lid will remain in the open position until it is closed. The CLARIOstar cannot start a test run if the lid is in the open position. Always make sure to close the lid before starting a test run.

5.5.3 Needle Holder

The needle holder houses two needles connected by tubing to the respective reagent pumps (Figure 17). The needles allow injection from reagent pumps into microplate wells.

The needle holder is removed from its magnetic cradle by gently pulling it away from the instrument. To perform priming, either (1) snap the needle holder into the park position (see Figure 22) or (2) hold the needle holder in hand. In either case, place a collection beaker or vessel underneath the needles for fluid collection.

To use the injection function of the needle holder, snap it into its magnetized working position (see Figure 16). If using an aperture spoon, attach the spoon to the needle holder before placing it into working position. (For instructions on attaching the aperture spoon, see Chapter 5.5.4)

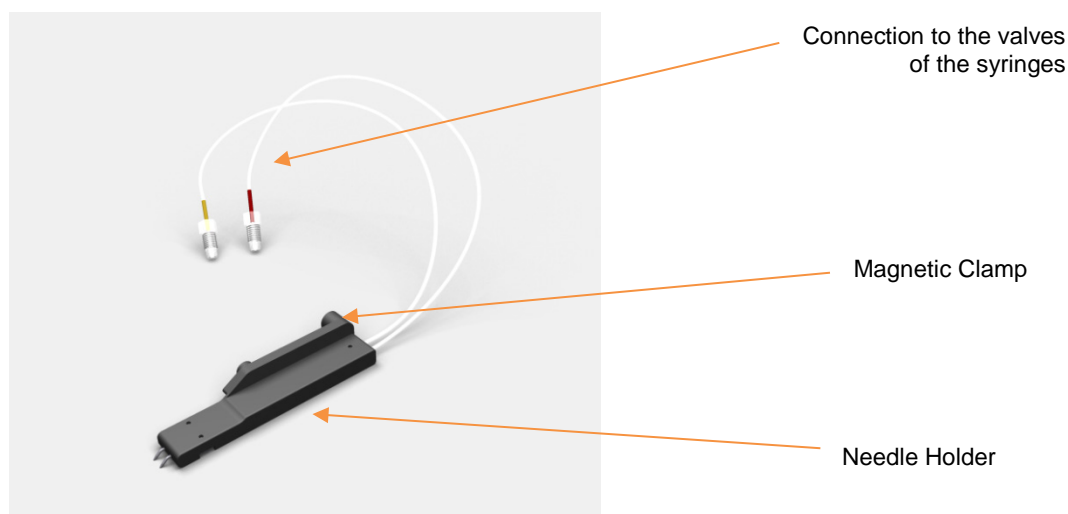


Figure 17: Needle Holder assembly

5.5.4 Aperture Spoon

Though the CLARIOstar already possesses superior crosstalk reduction as it reads individual wells, it is possible to reduce crosstalk even further in certain applications using aperture spoons to reduce even more ambient light from surrounding wells.



Figure 18: Aperture Spoons

Note that the aperture spoons should be used only with 384 and 1536 well plates and only in AlphaScreen[®] and luminescence applications. The aperture spoons are magnetized and snap on to the needle holder's right side, as shown in Figure 19.

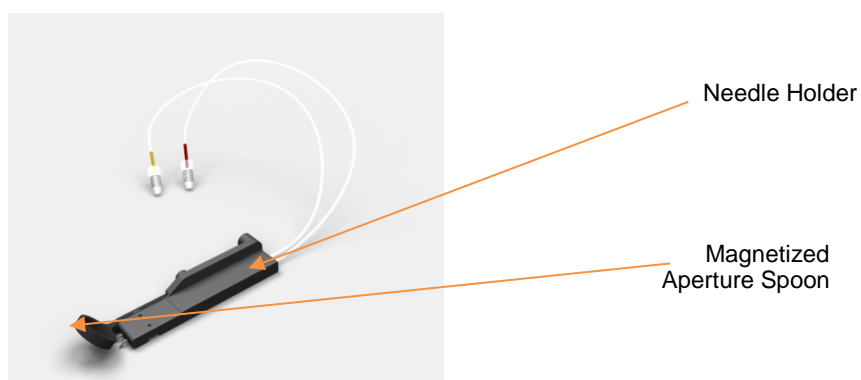


Figure 19: Needle Holder with Aperture Spoon attached

To install, choose the appropriate aperture spoon (labeled 384 or 1536) from the corresponding aperture holder located under the lid of the CLARIOstar and to the left front of the instrument (see Figure 2). Then snap the aperture spoon onto the right side of the needle holder. It is then possible to place the needle holder with aperture spoons into the working position (see Figure 16). The needle holder with aperture spoon attached fits into both park and working positions.

To remove the aperture spoon, snap it off of the needle holder and replace it to the aperture holder (Figure 20).



Figure 20: Aperture Spoon, placement in Aperture Holder

5.5.5 Holder for Reagent Compound Vessels

BMG LABTECH equips the CLARIOstar with a compound vessel holder (Figure 21). It accommodates the most commonly used compound storage tubes.



Figure 21: Compound Vessel Holder

5.5.6 Pump Priming

Pump priming is necessary to fill the tubing and needles of the reagent system with solution prior to executing a test run. Priming helps to avoid bubbles, which could lead to false results. Priming is also used to clean the tubing and to check that the solution comes straight out of the needle tips. If the fluid stream comes out at an angle this indicates that some debris is partly clogging the needle tip. In this case, cleaning of the tubing and the needle is required. Refer to Chapter 5.5.1 for the cleaning procedure.

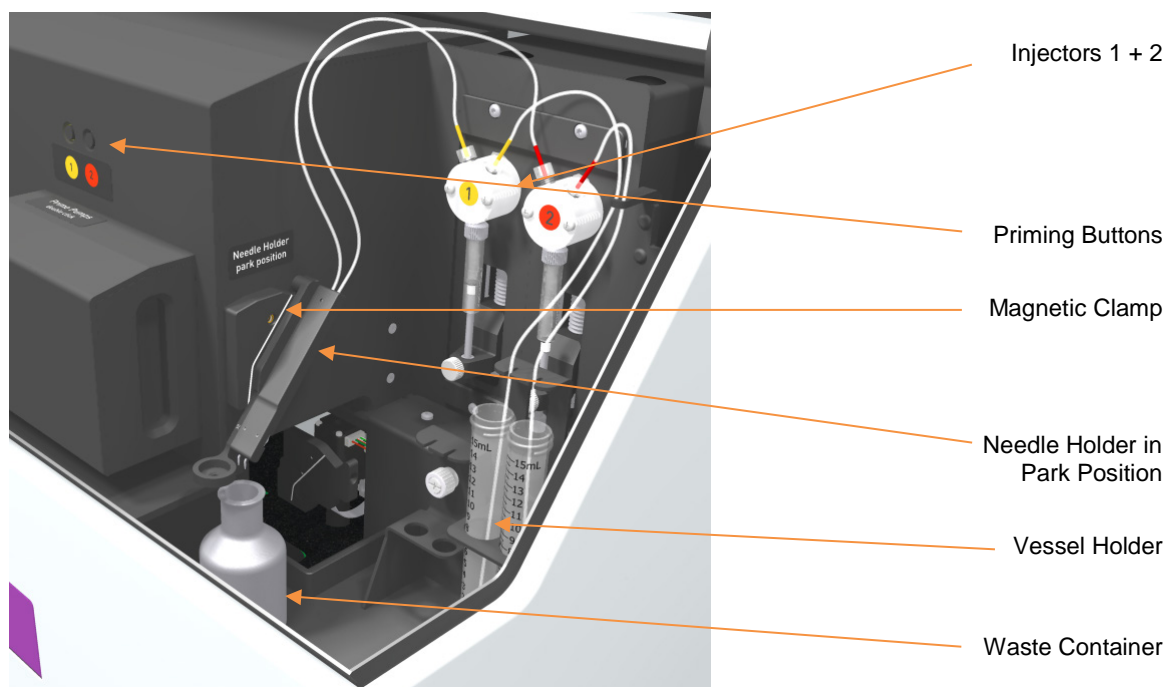


Figure 22: Internal Priming Conditions

Priming requires that the needle holder is placed in the park position (Figure 22) or held in hand and a waste container placed below the needle holder for fluid collection.

To start priming, place the waste container under the needle holder, a compound solution into the compound vessel, and insert the tubing into the solution. Priming is then initiated either via the control software or by a double click of the respective color-coded priming button.



It is important to pay attention while injectors are priming to ensure no solution spills inside the instrument. Additionally, check for a straight fluid stream during priming to ensure that needles are not clogged.

5.6 Automatic Height Sensor

The CLARIOstar measures the focusing in steps of 0.1 mm for both top and bottom reading modes. To perform the focus alignment (also known as Z-focus) as quickly as possible, an initial plate height monitoring takes place upon every plate-in command. For more information on the Z-focus, see the CLARIOstar software manual.

5.7 CLARIOstar - Stacker Connection

There are two connectors on the back side of the CLARIOstar, one for a PC and one for a Stacker.

The respective connection cables are provided with the instrument. The USB cable will connect the PC to the CLARIOstar ('Connection to PC') whereas the male-to-female RS232 cable (included with the Stacker) connects the CLARIOstar to the Stacker ('Connection to Stacker'). Cables and connection ports are not interchangeable.

The Stacker, when connected, will automatically be detected by the CLARIOstar, even if the Stacker is turned off.

The CLARIOstar can be operated as a stand-alone plate reader also when connected to a Stacker, as the plate carrier can be reached through the opening of the Stacker.

Both the CLARIOstar and the Stacker must be turned on when the reader is connected to the Stacker (Figure 23).

To use the reader with single plates, remove the magazines and the plate carrier of the reader can be accessed and used by pressing the plate in/out button or by using the control software.

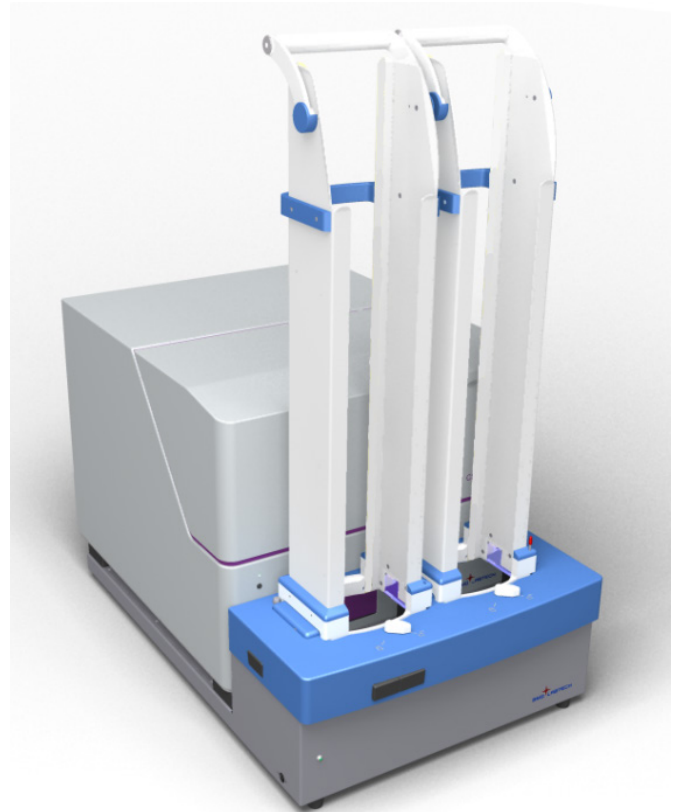


Figure 23: CLARIOstar with Stacker

6 Troubleshooting

6.1 Cleaning the Dust Filters

To avoid overheating of the CLARIOstar, it is necessary to clean dust filters periodically. In a normal laboratory environment, BMG LABTECH recommends cleaning dust filters once per year.

To clean the dust filters, turn off the main power, then snap the air inlet covers off the back of the instrument (see Figure 4) using your fingers. Remove the white foam dust filters and wash them in a mild soapy water solution and allow them to dry completely before reinstalling them. The metal filters found inside the dust filter inlets may also be cleaned in the same way if necessary.

6.2 Locking the Plate Carrier Manually

Check that the plate carrier is fixed by opening the plate carrier door with a fingernail and gently trying to move the plate carrier in all directions (Figure 24). If the plate carrier cannot be moved, it is locked. If the plate carrier can be moved, it is not locked. It is possible to lock the plate carrier manually, as described below:

- 1) Start with the transport pin in its unscrewed position, open the lid and open the plate carrier door.
- 2) Position the plate carrier with the right hand at the same time as the transport pin is moved down with the left hand. First push the plate carrier gently to the back left corner inside of the instrument, then move the pin gently down. It may be necessary to move the plate carrier around slightly until the transport pin is guided by the guide groove (Figure 25). When pin is in the groove move plate carrier to the front until pin snaps down into the thread.
- 3) Turn the pin clockwise until it is fixed tightly.



Figure 24 : With a fingernail, open the plate carrier door and see if the plate carrier is locked by gently trying to move it

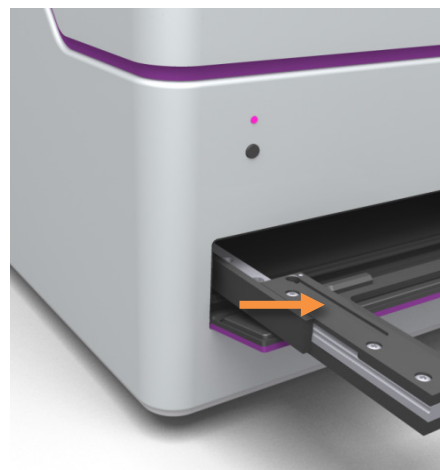


Figure 25: The groove in the plate carrier guides the transport pin