NEPHELOstar *Plus*

Operating Manual

Revision B

The NEPHELOstar Plus is a fully automated laser-based microplate nephelometer that measures forward light scattering. The instrument can be used for a wide range of applications including solubility testing, bacterial growth, immunoprecipitation, quality control, and HTS drug development. The key feature of the robust optical system is a feedback-controlled laser that offers adjustable intensity and beam diameter to reduce meniscus effects and optimize sensitivity. Instrument flexibility is further enhanced by two programmable syringe injectors (optional), an incubator, and fully programmable plate shaking. It is designed to read a variety of microplate formats up to 384 wells.

NEPHELOstar *Plus*

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This manual is designed to guide NEPHELOstar Plus users through the basic hardware features of the instrument.

Although these instructions were carefully written and checked, we cannot accept responsibility for problems encountered when using this manual. Suggestions for improving this manual 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 www.bmglabtech.com or send an email to germany@bmglabtech.com.

Intended Use

The NEPHELOstar Plus microplate reader is intended for professional laboratory research use by trained personal who understand the nature of photometry.

The instrument may be used only for research and development or other non-clinical purposes.

For validation of the entire system it is recommended that Good Laboratory Practices (GLP) are followed to ensure reliable analyses

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1 Technical Specifications

Light Source
- Self monitoring laser diode
- Wavelength 635 nm
- Selectable beam width: 1.5-3.5 mm
- Selectable intensity in 100 steps

Reagent Injection
- Up to 2 built-in reagent injectors
- Injection at measurement position
- Individual injection volumes for each well (3 to 350 µL)
- Variable injection speed up to 420 µL / s
- Up to four independent injection actions per well
- Reagent back flushing

Shaking
- Linear, orbital, and double-orbital shaking
- User-definable time and speed

Incubation
- Incubation range from ambient +4°C to 45°C in 0.1°C steps
- Temperature stability 0.2°C
- Temperature monitoring (without incubation)

Plate Carrier
- Auto lock microplate carrier
- All microplate formats up to 384-well
- Microplates should fulfill the SBS specification and non-SBS formats should fit: (lxwxh) (mm) max: 128x86x22; min. length 124

Reading Time
- 16 s for 96 well plate (shortest possible time)
- 47 s for 384 well plate (shortest possible time)
  depends on assay conditions and liquid surface stability

Sensitivity
- Silica detection (size 0.5 – 10 µm) sensitivity: 800 nM

Dynamic Range
- 5 decades

Computer Interface
- USB 2.0

Dimensions
- Height: 32 cm, Width: 44 cm, Length: 48 cm

Weight
- 28 kg (including 2 pumps)

Power
- 100-240 V, 50/60 Hz, max peak 300 VA
- Consumption:
  Instrument off: 0 W
  Normal operating conditions: 15 W
  Normal operating conditions, incubation set to 37°C: 50 W
- Fuses: T 5A/250V (use original spare fuses only)

Ambient Conditions
- Operating Temperature: 15 °C to 35 °C
- Storage Temperature: -10 °C to 50 °C
- Humidity of atmosphere: 20 % to 80 %
- Non-condensing

Instrument Conformity
- Overvoltage category II
- Contamination class II
- Protection class I
- Laser class I

Options
- Stacker for 50 microplates
- ACU: Atmospheric Control Unit to regulate both O_2 (1-19%) and CO_2 (0-20%) inside the microplate chamber
- Gas Vent: Passive purge gas vent to replace the atmosphere in the microplate chamber
- Fan Control: improved control of microplate ambient conditions, especially for long-term incubation experiments

Limit of detection was calculated according to the IUPAC standard: 3 x (SD_{blank})/slope
Specifications are subject to change without notice.

2 Safety Information

2.1 Description of warnings

The warning signs used throughout this manual adhere to the description set forth in DIN 4844-2.

A general warning calls attention to a condition which is further described and must be strictly followed by the operator.

Warning for optical radiation

Warning for laser beam

2.2 General Information

This instrument must be installed and used as lined out in this Operating Manual. Installation, service and any operation which requires opening 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 vapour. The surface of the table should be flat and even. Avoid areas subject to vibrations and direct sunlight

Prior to turning the instrument on the first time let the instrument adapt to room temperature for at least 3 h to avoid condensation causing a short circuit.
BMG LABTECH will void the warranty if damage occurs to electrical and/or mechanical parts in case 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

Samples and reagents, be it 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 an earthed supply socket. The instrument is class 2 construction and must be earthed.
- 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 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 reagent door during a measurement run.

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

The following light source is incorporated and encapsulated in the NEPHELOstar Plus:

Self-monitoring laser diode – laser class 2. Emits light at a continuous wavelength of λ=635 nm with <1 mW and will not injure the eye if exposure is limited to max. 0.25 sec. Nevertheless, 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 solid state laser. The instrument is rated a class 1 laser product.

2.6 Chemical and Biological Safety

Daily routine 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, be it 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.

Gas vent connection – for the standard gas purge vent use a regulator followed by a flow restrictor to set the flow rate. The instrument should be housed in an atmospheric gas tight bag (e.g. Aldrich® AtmosBag, Z530220-1E) to prevent spillage of gas

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

2.7 Cleaning and 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 of the operating manual of the instrument.

Name: _____

Company: _____

_____

_____

_____

Date, Signature: _____

3 Installation

When unpacking the instrument, please check to ensure that all of the following parts are included:

- **NEPHELOstar Plus** reader
- Control and MARS Data Analysis software (CD-ROM)
- Manual
- Power cord
- USB cable
- Accessories: - 2 extra fuses: 5A/250V for main power 100V-240V
- Accessories for instruments with reagent injectors:
  - 4 spacers
  - Injection head (installed in instrument)
  - Injector needle cleaner
  - closure for measurement position

Call BMG LABTECH immediately if any of these items are missing.

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

After unpacking and positioning the reader, please do the following steps in the given order:

1 – Release transport locks
2 – Install software
3 – Plug in power and USB connection

Always install the software before plugging in USB connection!

3.1 Transport Locks

When the instrument is shipped or moved to a different location, the transport locks should be in the locked position. Lock and unlock the transport locks only when the instrument is not powered on.

3.1.1 Microplate Carrier Transport Lock

The microplate carrier transport lock is located in the back left corner of the reagent box (figure 1 and figure 7). Once the instrument is in its permanent location, the transport pin should be unlocked to free the plate carrier. To do this turn the transport pin counter-clockwise until it is moved up by the spring.

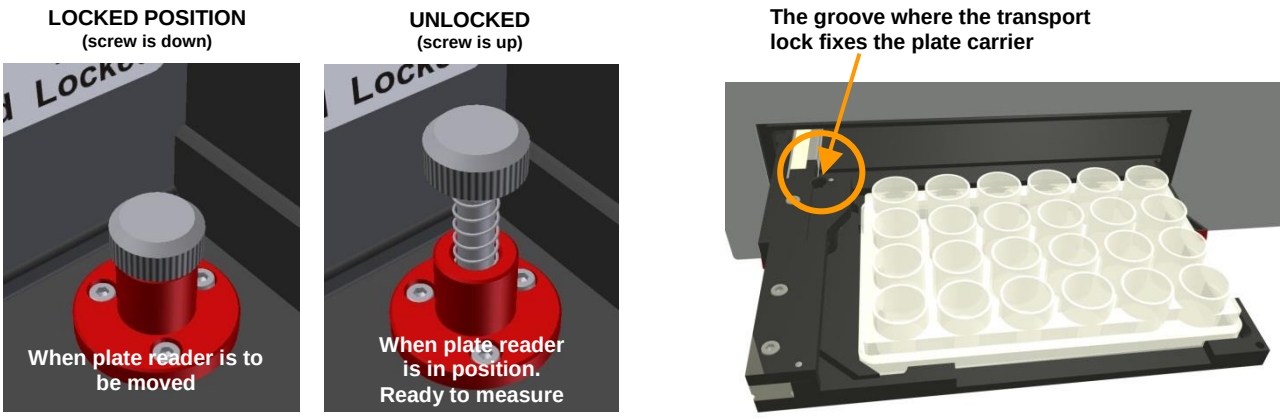


Figure 1: Left: transport lock in locked position (screw is down).
Middle: transport lock in unlocked position (screw is up).
Right: the groove where the transport pin can lock the plate carrier

If the NEPHELOstar Plus needs to be moved to a new location, the plate carrier should be in the locked position otherwise the transport system could be damaged.

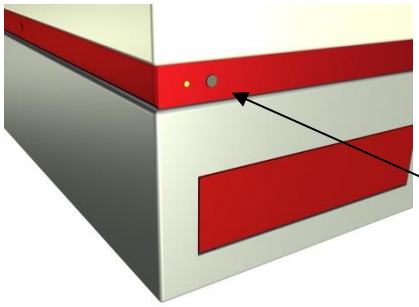


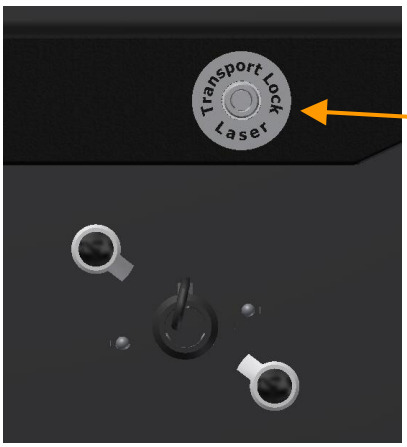
Figure 2: Plate In / Plate Out button.

Press and hold the plate in / plate out button for 3 seconds, hereafter the plate carrier will automatically move to its lock position. Once the reader is switched off, the transport pin can be moved down and turned clockwise. The transport pin must be screwed until it tightens. Please tighten it firmly with your fingers. No tools are required.

The microplate carrier system is locked when the transport lock is in its down position and firmly tightened.

3.1.2 Laser Transport Lock

The NEPHELOstar Plus laser must be secured and locked for transportation or moving the instrument. The laser is mounted on a freely moving z-axis and a spring-loaded screw secures the z-axis in the locked position. Rotate the spring-loaded screw counter clockwise by hand to unlock the laser. Once the screw is lose move the z-axis with the laser slightly down to check if it is free. To secure the laser in the transport lock position, move the z-axis up until it touches the top. Hold the z-axis firmly while rotating the spring-loaded screw clockwise to secure the laser in the lock position.



Rotate the spring loaded screw counter clockwise to unlock, and clockwise to lock the laser in the transport position

Fig. 3: Top view of the laser transport lock screw in the reagent box

3.2 Software Installation

Before connecting the instrument's USB communication cable the software must be installed! Please follow the instructions in the software manual.

3.3 Power and Communication Connections

- **Power Connection**

First check that the power switch on the back of the instrument is in the "Off" position. Inspect the voltage information on the label next to the power switch to ensure that it corresponds to the local main power specifications. Also make sure the power cable is grounded. Hereafter, the power cable can be connected to the instrument.

- **USB Communication Connection**

Connect the USB cable to the NEPHELOstar Plus and to the USB port on the PC. Please connect the reader directly to your PC and do not use a USB-hub.

Only connect a computer that corresponds to EN 60950 and UL 1950 for data processing instruments.

You can perform a connection check within the setup menu of the Omega - NEPHELOstar Plus software (go to Omega / Settings / and click ‘Connection check’).

If the instrument and PC are communicating, a ‘Connection OK’ message will appear.

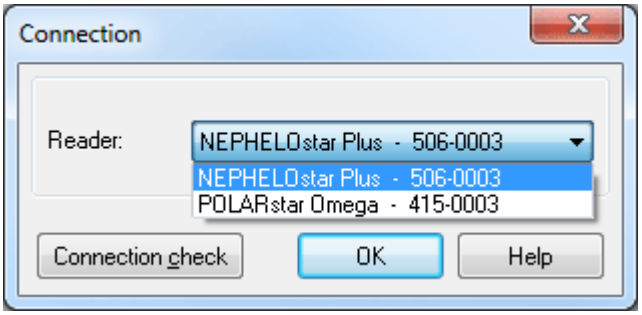


Figure 4: Connection check window (‘Omega / Setting / Connection’)

4 Instrument Overview

Front View

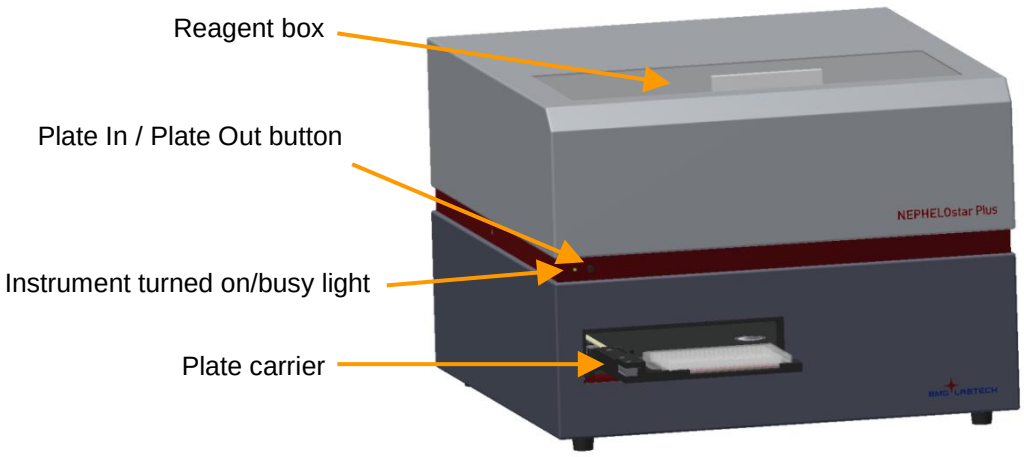


Figure 5: NEPHELOstar Plus

A constant green light means the instrument is turned on. A flashing green light means the instrument is busy (e.g. performing a measurement, plate in/out, priming, etc.). A faster flashing green light (5 flashes per second) means an error has occurred.

Back View

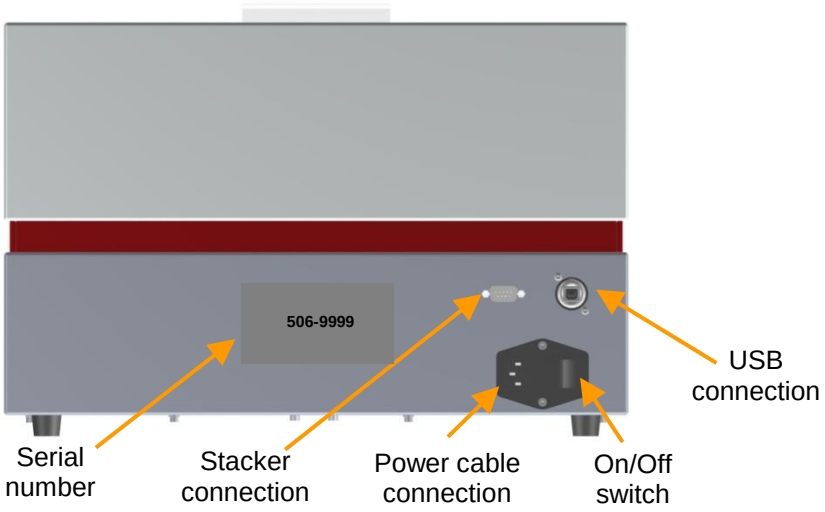


Figure 6: Back of NEPHELOstar Plus reader

Top View, Reagent Box

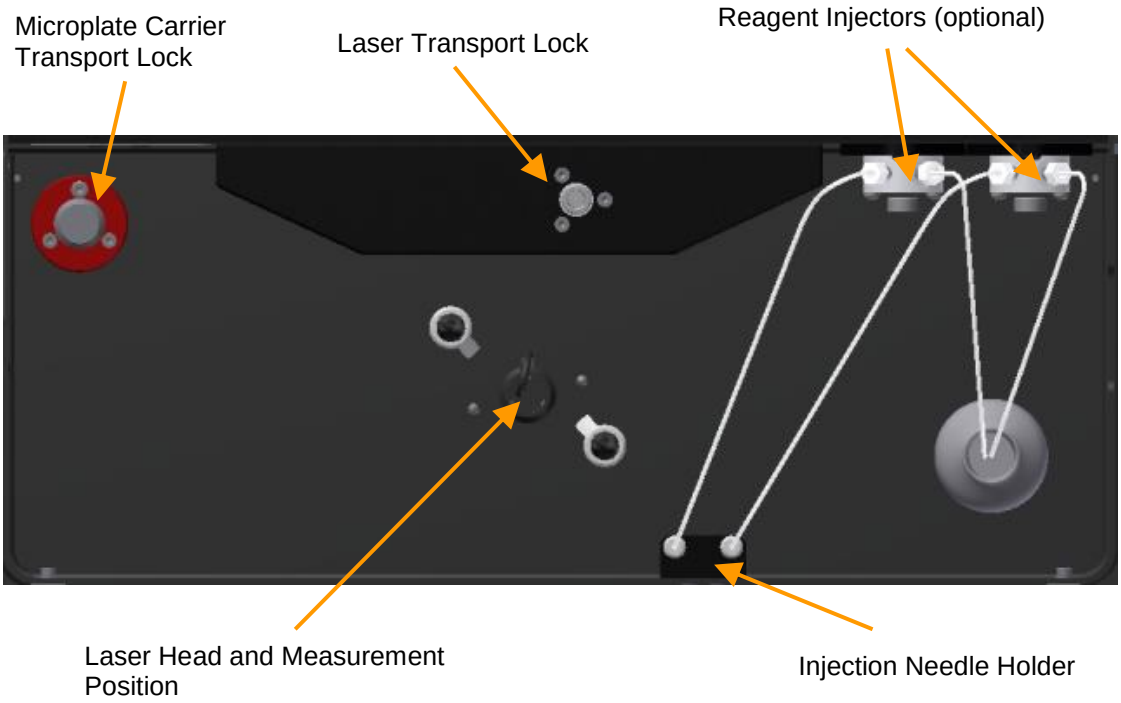


Figure 7: NEPHELOstar Plus - top view of reagent box

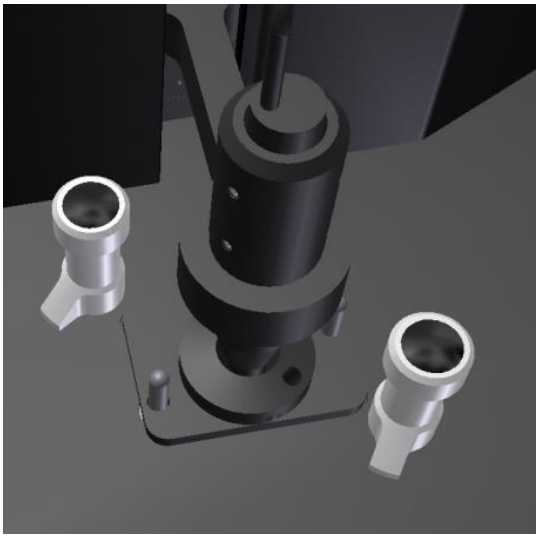
5 Description of Components

5.1 Laser Guidance

The NEPHELOstar Plus reader is equipped with a self-monitoring laser and the laser beam is guided through a quick-fix mounting for top reading. Depending on if the NEPHELOstar Plus is equipped with / without injectors a different mounting is installed. If a mounting with holes for the injection needles need to be installed the original mounting needs to be exchanged.

If spacers for microplate format of < 96 well need to be installed (see section 5.2), the quick-fix mounting needs to be removed to position spacers underneath. No tools are required. Lift up the quick-fix knob by hand and turn the nose away from the quick-fix mounting. Remove the mounting and place spacers and the other quick-fix mounting through the positioning pins. Lift up the quick-fix knob and turn the noise towards the mounting and release the knob to fix the mounting.

Quick-fix: in released position



Quick-fix: in fixed position

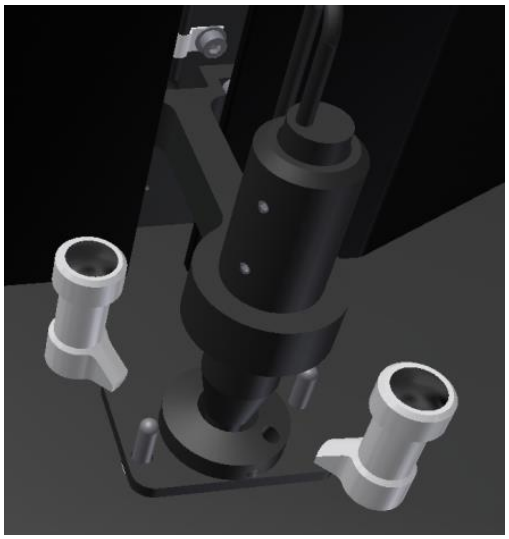


Figure 8: Quick-fix - pull up and turn to change injection head

5.2 Spacers

The NEPHELOstar Plus reader is designed for most microplate formats. The height of some microplates exceeds the space allowed under the injection head. The minimum space between the injection head and microplate should be 1.5 mm. With 6, 24, 48-well plate formats, it will be necessary to raise it using the spacers provided in the service box.

The spacers are metal rectangular pieces with a hole in the center. Each spacer is 2 mm in height. They will be installed between the laser head and the bottom of the reagent box. The number of spacers used depends on how high the injection head needs to be elevated.

Determination of the number of spacers:

If the height of the microplate exceeds the height of the left border of the plate carrier, (see figure 9) spacers need to be installed under the injection head (see figure 10). There should be enough spacers so that the height of the left side of the plate carrier is slightly higher than the microplate.

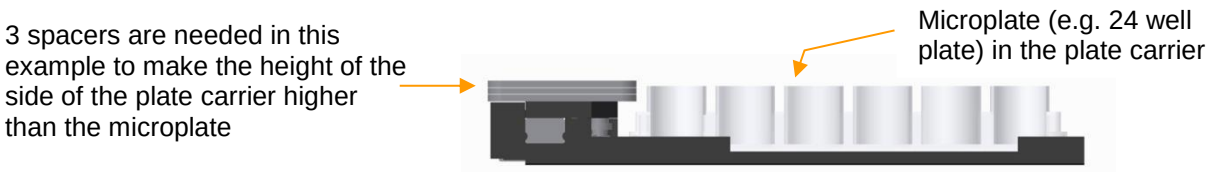


Figure 9: Front view of the plate carrier

Installation of spacers:

If you install spacers, first remove the injection needles (if any) and then remove the quick-fix mounting. Install the appropriate number of spacers using the positioning pins as a guide. Reinsert the quick-fix mounting and secure it.

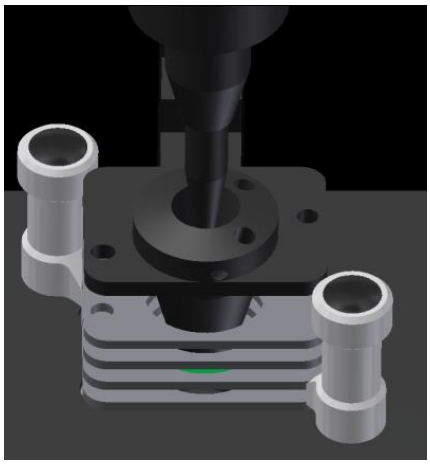


Figure 10: Example of spacers between injection head and bottom of reagent box

As a cross check (to ensure that the microplate can pass under the head), push the plate carrier manually into the instrument and slowly move it towards the optic. If there is approximately 1.5 to 2 mm of space between the optic and microplate, then enough spacers were installed.

5.3 Reagent Injectors

The NEPHELOstar Plus reader can be equipped with up to 2 reagent injectors (figure 11).

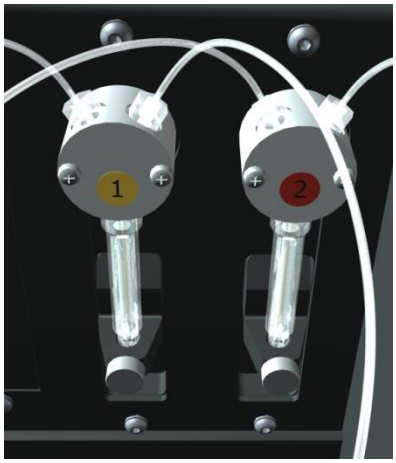


Figure 11: Reagent injectors

When the reagent injector(s) are not in use, the needle(s) can be placed in the needle holder (figure 7).

The reagent needles are made of stainless steel, the tubings 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 chemical resistant materials that are available.

The needle tip plays a major role regarding the pumps' accuracy. Always treat the needles with care. That is, be careful when positioning the needles in the measurement head or in the needle holder.

For obtaining optimal performance of the reagent injectors, please see the following chapter.

Note: Before using a pump in a test run, you need to prime this pump. This can be done using the Control software (menu command ‘Measure | Prime’). You can also prime the pumps without using the software. When you open the reagent door, you will see two buttons on the left side. To prime a pump double click the respective button.

5.3.1 Use and Maintenance of the Reagent Injectors

To remove cellular debris and viscous solutions from the syringe barrel:
Take off the syringe barrel and rinse it with distilled water. It may be useful to use the wire syringe cleaners (found in the service box) to scrape particles off the walls.

In order to obtain optimal performance from the reagent injectors, it is recommended to follow these guidelines in the use of the reagent injectors:

- **Do not use the syringes more than two cycles without liquid.**
- **After each use, thoroughly flush the syringes with distilled water.**
- **If the plunger is removed from the syringe barrel, it should be wiped with ethanol before replacing.**
- **Syringes should be cleaned each week using one of the following procedures:**

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 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 the solution in the syringe for 10 minutes.
4. Flush the syringe a minimum of 10 times with distilled water.