

Photobioreactor FMT150

Manual and User Guide

Please read this manual before operating the product.



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1. WARNINGS AND SAFETY PRECAUTIONS

PLEASE READ THE FOLLOWING INSTRUCTIONS CAREFULLY BEFORE TURNING THE PHOTOBIOREACTOR ON:

- Remove all packaging and transport protectors before connecting the photobioreactor to the power supply.
- Use only cables supplied by the manufacturer.
- Keep the device dry outside and avoid working in high humidity environment!
- The manufacturer is not responsible for any damage due to improper operation!
- Water and other liquids should only be placed in vessels designed for the purpose and according to instructions included in this manual.

GENERAL ELECTRICAL SAFETY GUIDELINES:

- Perform a routine check of the devices and their wiring.
- Replace worn or damaged cords immediately.
- Use appropriate electrical extension cords/power bars and do not overload them.
- Place the device on a flat and firm surface. Keep away from wet floors and counters.
- Avoid touching the device, socket outlets or switches if your hands are wet.
- Do not perform any alterations to the electrical parts of the device or its components.

Warning:

The Photobioreactor is considered Class 1M* LED Product. LED radiation may be harmful to eye. Avoid direct and strongly reflected exposure. Use protective glasses.

**Class 1M: Laser and LED equipment that is safe for the naked eye under foreseeable conditions of operation. Looking directly into the source of radiation by employing optics within the beam, such as magnifying glass, telescope or microscope, can be potentially hazardous.*



2. GENERAL DESCRIPTION

Photobioreactor FMT150 feature a unique combination of the cultivator and computer controlled monitoring device. The Photobioreactor FMT150 combines a cultivation vessel with the built-in fluorometer and densitometer. It is primarily designed for modular and precise phototrophic cultivation of algae, cyanobacteria and bacteria. Light power and spectral composition as well as temperature and aeration gas composition can be set with high accuracy.

User defined cultivation conditions can be dynamically varied according to user defined protocol. Continuous online monitoring of cultivation conditions and measured parameters is possible via the optional PBR software. Light, temperature, gas composition, and culture medium regime can be programmed to oscillate with various amplitudes and frequencies with user-defined time steps from milliseconds to hours via the software or via the control unit. Turbidostatic cultivation can be used for the stabilization of the suspensions via optical density control. In addition, up to 8 peristaltic pumps for different chemostat cultivation control can be linked to the Photobioreactor FMT150 for the highly precise control of cultivation conditions.

The growth of the cultures is monitored by the integrated densitometer (OD 720, 680 nm). Relative chlorophyll content of the culture can also be monitored continuously by the difference of optical densities at 680 and 720 nm. The instantaneous physiological state of the culture is measured by the Photosystem II quantum yield F_v/F_m .

The Photobioreactor FMT150 has a flat-vessel design that enables uniform illumination over the whole culture. Photobioreactors are currently manufactured in three cultivation vessel capacities: 400 ml, 1000 ml and 3000 ml.

All Photobioreactor models may be supplied with a number of optional accessories – sensors, stirrers, pumps, etc. Control of the Photobioreactor is possible with either the control unit of the photobioreactor itself or the PBR software. However, the PBR software provides much wider functionality to define, control, display and analyze the experimental conditions and data.

3. DEVICE DESCRIPTION

Standard version of the Photobioreactor FMT150 consists of a cultivation vessel with a sealable lid and a base box containing electronics circuitry, LED light panel (Fig. 1A) and other components essential for the optimal operation of the photobioreactor.

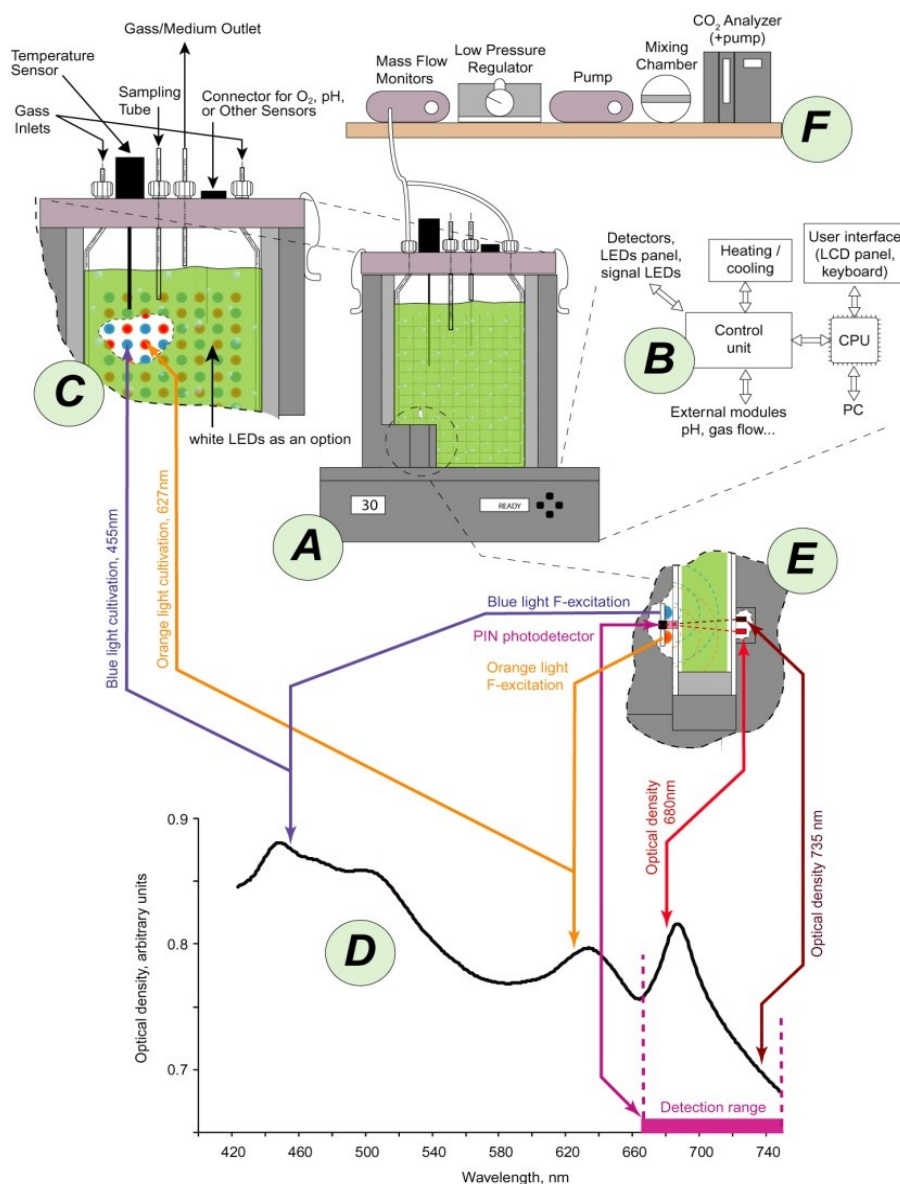


Fig. 1: A) Photobioreactor body consists of a flat vessel holding the suspension and of a base box with electronics circuitry. B) The block scheme of the instrument shows the Control Processor Unit exchanging data and protocol with external computer (PC) and auxiliary external modules. The Control Unit governs measurements of parameters such as OD and chlorophyll fluorescence plus parameters measured by auxiliary sensors. It also controls the actinic light sources and temperature of the culture. C) Detail of the LED configuration. D) Uncorrected optical density spectrum of cyanobacterial suspension. The lines with arrows indicate wavelengths of various light sources that are integrated in the instrument. The detection range of the photodiode is also shown. E) Detail (rotated top-to-bottom view) of the fluorometer and densitometer. F) Schematic representation of optional gas supply components and modules that can be used to stabilize aeration and set CO₂ concentration level.

3. Device Description

The array of high-power light emitting diodes (LEDs) is located behind the cultivation vessel.

In the bottom corner of the front of the vessel is a semiconductor light sensor for measuring fluorescence emission and suspension optical density by attenuation of light that was emitted from the LEDs (Fig. 1E)

The Photobioreactor can also control external modules such as peristaltic pumps and solenoid valves via the photobioreactor control unit (shown schematically in Fig. 1F and Fig. 2). The solenoid valves are used to switch off the gas supply to the culture (bubbling) during optical measurements. Peristaltic pumps are used in turbidostat and chemostat modes to supply fresh medium or buffer.

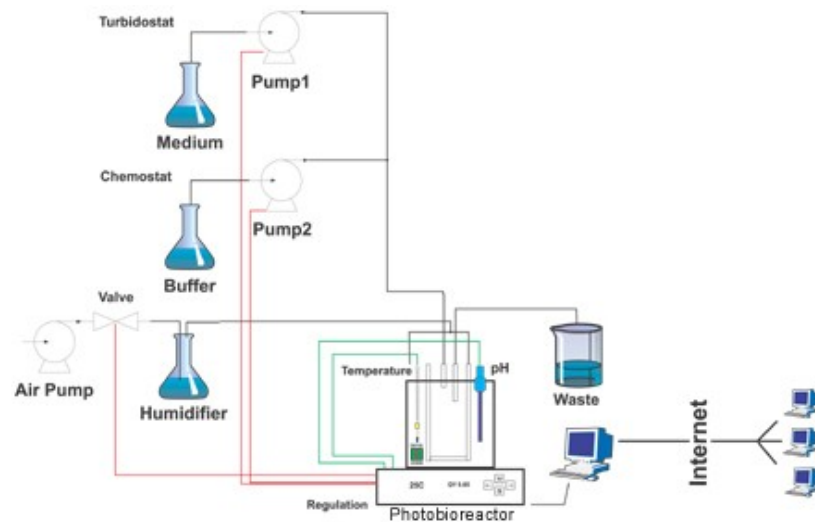


Fig. 2: Schematic diagram of the Photobioreactor, its modules, and remote control.

3.1 CONFIGURATIONS AND VERSIONS

FMT150 can be shipped with various configurations of vessel capacity, light quality, light intensity and thermoregulation. Numerous additional accessories such as various sensors can be supplied as described in chapter 5 on page 30.

Please note that this manual describes operation of two FMT150 versions (Fig. 3) - older PBR FMT150.1 and new PBR FMT150.2.

3.1.1 CULTIVATION VESSEL

The cultivation vessel is flat and rectangular in shape. Its front and back windows are made of glass plates. The base is made out of stainless steel and contains a thermal bridge that facilitates heat transfer between a Peltier cell in the instrument base and the culture suspension. The lid of the vessel is easily detachable during cleaning and contains Luer connectors for gas or medium supply tubing, waste medium exhaust or sample collection. The three larger ports accommodate the optional temperature/pH sensor and dissolved O₂ or CO₂ probes.

The Photobioreactor FMT150 is manufactured in three vessel capacities enabling the customer to select optimal cultivation conditions for his experiments.

- 400 ml (in FMT150/400)
- 1000 ml (in FMT150/1000)
- 3000 ml (in FMT150/3000)

3.1.2 LED LIGHTNING

The high-power light emitting diodes generate a highly uniform irradiance flux that can be controlled in the range 0-3000 $\mu\text{mol}(\text{photons})/\text{m}^2/\text{s}^1$ PAR. The irradiance can be dynamically modulated by the FMT150 control unit or by the PBR software.

Two **standard LED panel** versions are available, each colour is controlled independently:

- White-Red panel: maximum intensity 1,500 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$ (white 750 / red 750 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$)
- Blue-Red panel: maximum intensity 1,500 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$ (blue 750 / red 750 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$)

Two optional **upgraded LED panel** versions are available, each colour is controlled independently:

- White-Red panel: maximum intensity 3,000 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$ (white 1500 / red 1500 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$)
- Blue-Red panel: maximum intensity 3,000 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$ (blue 1500 / red 1500 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$)

Optional **customized panel** versions:

- White-Blue: maximum intensity 1,500 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$
- White-Blue with Light Upgrade: maximum intensity 3,000 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$
- Red: maximum intensity 750 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$ (no light upgrade available)
- White: maximum intensity 1,500 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$

3. Device Description

- White with Light Upgrade: maximum intensity 3,000 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$
- Blue: maximum intensity 1,500 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$
- Blue with Light Upgrade: maximum intensity 3,000 $\mu\text{mol}(\text{photon})/\text{m}^2/\text{s}$

3.1.3 THERMOREGULATION

It requires presence of the pH/Temperature probe.

- Standard range of 18 – 50 °C (static or ramping).

Please find approximate estimation of time required for the given temperature equilibration in the Table 1 below.

Initial temperature	Target temperature	Light conditions $\mu\text{mol}/\text{m}^2/\text{s}$	Equilibration time required
5 °C	50 °C	1500	2.5 hours
50 °C	5 °C	1500	3 hours

Table 1: Temperature equilibration times

- Optional enhanced range 0 – 70 °C (static or ramping) (requires enhanced vessel made from nickel-plated brass covered with ceramic layer).

3.1.4 VERSIONS

The available vessel capacity for two FMT150 versions:

- FMT150.1 – 400ml, 1000ml
- FMT150.2 - 400ml, 1000ml, 3000ml

Important: The FMT150 in both 400 and 1000 ml models has been upgraded from FMT150.1 (Fig. 3A) to FMT150.2 (Fig. 3B). This manual provides instructions for operation of both versions (original, now referred to FMT150.1 and the current version FMT150.2). Please always ensure that you refer to correct instructions.

3.Device Description

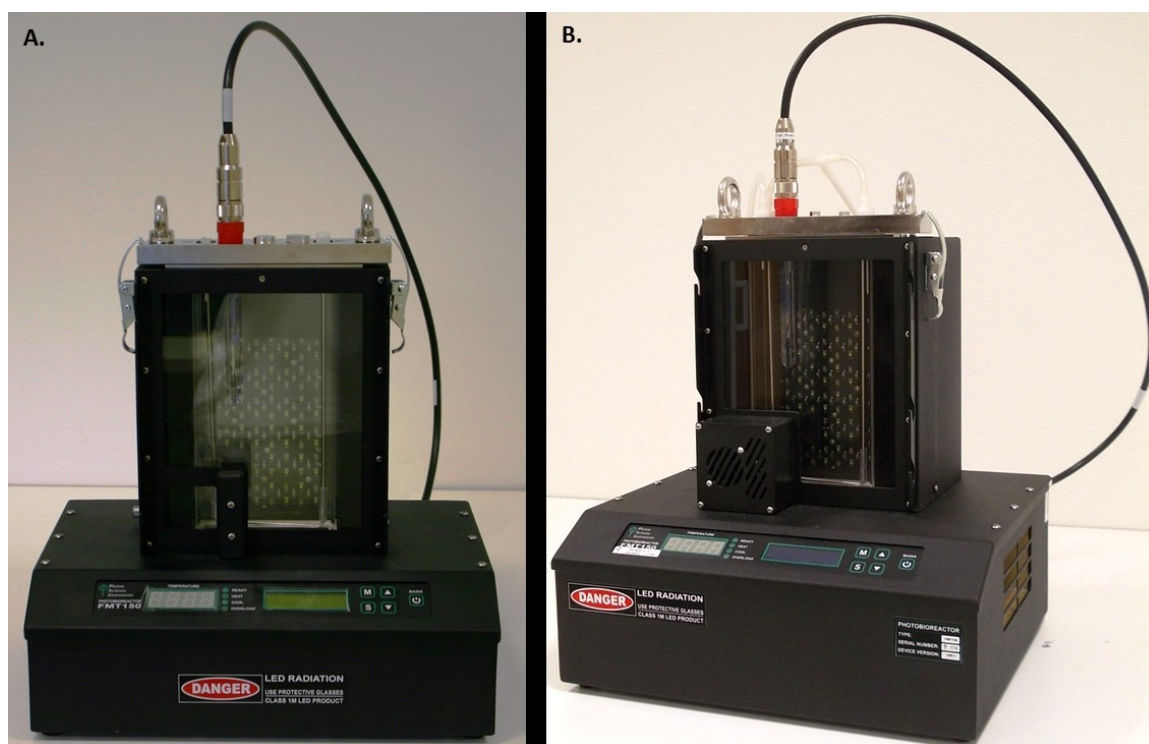


Fig. 3: Two versions of the PBR FMT 150: A) FMT 150.1, B) FMT 150.2.

3.Device Description

List of parameters measured and/or calculated by the Photobioreactor FMT150

Symbol	Name	Description
F_t (Dark)	Instantaneous fluorescence in the dark	Instantaneous F-yield in dark equals F ₀ F_t = F₀ (in dark-adapted sample)
F_t (Light)	Instantaneous fluorescence in the light	Instantaneous F-yield in light that results from a dynamic equilibrium of plastoquinone reducing and re-oxidizing processes and from non-photochemical quenching F_t = F_s (in light-adapted sample)
F_M (Dark)	Maximum fluorescence in dark-adapted state	Maximum F-yield with Q _A reduced (q _p =0) and non-photochemical quenching relaxed (NPQ=0)
F_M (Light)	Maximum fluorescence in light-adapted state	Maximum F-yield with Q _A reduced (q _p =0) and non-photochemical quenching active (NPQ≠0)
F_V	Maximum variable fluorescence	F_V = F_M - F₀
QY (Dark)	Instantaneous PSII quantum yield in the dark	Effective PSII quantum yield QY= F_V / F_M (dark-adapted sample)
QY (Light)	Instantaneous PSII quantum yield in the light	Effective PSII quantum yield relaxing in light QY= F_V' / F_M' (light-adapted sample)
OD₆₈₀	Optical density at 680 nm	Optical density representing light scattering and chlorophyll absorption.
OD₇₂₀	Optical density at 720 nm	Optical density representing light scattering.
pH	pH	pH is the negative logarithm of the hydrogen ion concentration in the medium.
[O₂]	Oxygen concentration	Concentration of dissolved oxygen.
[CO₂]	Carbon dioxide concentration	Concentration of dissolved carbon dioxide. CO ₂ partial pressure is calculated using the measured pH and the temperature.

Table 2: FMT150 measured parameters

3.2 STANDARD COMPONENTS

Photobioreactor FMT150 can be purchased in different configurations from minimal up to complex system consisting of numerous accessories linked together. They can be controlled via the Photobioreactor control unit itself or by the PBR software for full operation of the system. However, the Photobioreactor control unit offers only static manual control.

Minimal required configuration that is recommended to be purchased for optimal operation of the Photobioreactor cultivation and monitoring system consists of **set of standard photobioreactor components** (as listed below) plus air pump with bubble interruption valve and **pH/temperature module**. Components that are part of minimal Photobioreactor FMT150 configuration are shown in Fig. 5. Optional components that are available as accessory modules and can be purchased together with Photobioreactor FMT150 are described in chapter 5

Photobioreactor FMT150 purchased in minimal configuration can be effectively used for cultivation and monitoring purposes, however without purchasing PBR software no measured data can be stored and visualised as well as no user-defined protocols can be designed. Chapter 3.3 and chapter 8.1 describe how to operate the PBR FMT150 in cultivation mode via front panel control unit, without use of PBR software.

Note: Customers are kindly asked to check the contents of the delivered package and compare it with enclosed package list generated based on customer's order. Please contact the support@psi.cz if any discrepancies occur.

As shown on Fig. 5 on page 14 the FMT150 is shipped with:

1. **Power supply with the power cord.**
2. **Photobioreactor with integrated double-modulation fluorometer**- Ft, Fm, QY and integrated optical density measurement.
3. **Cultivation vessel** with capacity 400 ml (with holder) for FMT150/400, 1000 ml for FMT150/1000 or 3000 ml for FMT150/3000.
4. **LED panel shield.**
5. **Spare Part Kit*** – for the complete list of Spare Part Kit see Table 3 on page 13 and corresponding Fig. 4
6. **User guide (this document) and Software manual.**
7. **Bubble Humidifier** – To maintain stable culture volume.

Components #8 and #9 below are highly recommended for proper functionality of the PBR, but are not included in the standard configuration and have to be purchased separately.

8. **Bubble interruption valve with air pump** (air pump not shown on Fig. 5).
9. **pH/temperature module.**

3.Device Description

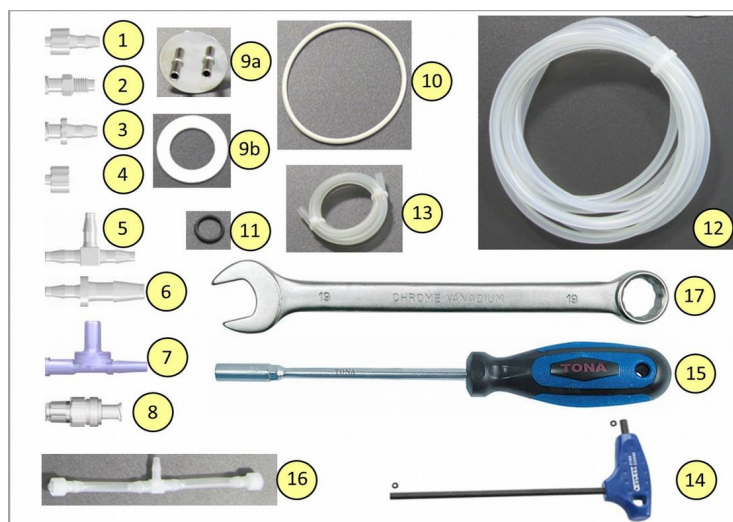


Fig. 4: Components of the spare part kit. Numbers correspond to component numbers in Table 3.

Component Number	Component Description	Specification	Explanation	Pcs
1	Male Luer Integral Lock MTLL240-J1A*	5/32" (4.0 mm)		10
2	Female Luer Thread Style BSFTLL-J1A*	5/16" Hex to 1/4-28 UNF Bottom Sealing Thread		10
3	Female Luer Thread Style FTLL240-1*	5/32" (4.0 mm)		10
4	Male Luer Integral Lock Ring Plug LP4-J1A*			10
5	Tee Tube Fitting T40-J1A*	5/32" (4.0 mm)		10
6	Reduction Tube Fitting 4060-1*	1/4" (6.4 mm) and 5/32" (4.0 mm)		5
7	Double Check Valve DCV118-001*		Safety valves that permit gases and liquids to flow in only one direction	5
8	Check Valve SCV08053*		Check valve for with low priming volume	5
9	Metal lid with ports (9a) with silicone ring (9b)	Silicon ring 27x42x2 mm	For humidifier bottle	1+2
10	O-ring	75x3 mm	Sealing between lid and the vessel	5
11	O-ring	14x2 mm	Sealing to the openings for optional sensors	10
12	Silicon Tubing	Ø 6/3 mm	Additional tubing	10 m
13	Silicone tubing	Ø 5/2 mm	Additional tubing	0.5 m
14	Hex Key	5 mm	To loosen up the screws of the lid	1
15	Spanner/Screwdriver	8 mm	For releasing filler screws in optional sensor positions and luer connectors in the vessel lid	1
16	T-tube (silicone tubing with 2 pcs of Male Luer Lock and 1 pc of Tee Tube Fitting)		Aeration tubing for the vessel	1
17	Wrench	19 mm	For releasing sensors	1

Table 3: List of spare part kit components shown in Fig. 4

* Provided by: www.valueplastics.com

3.Device Description

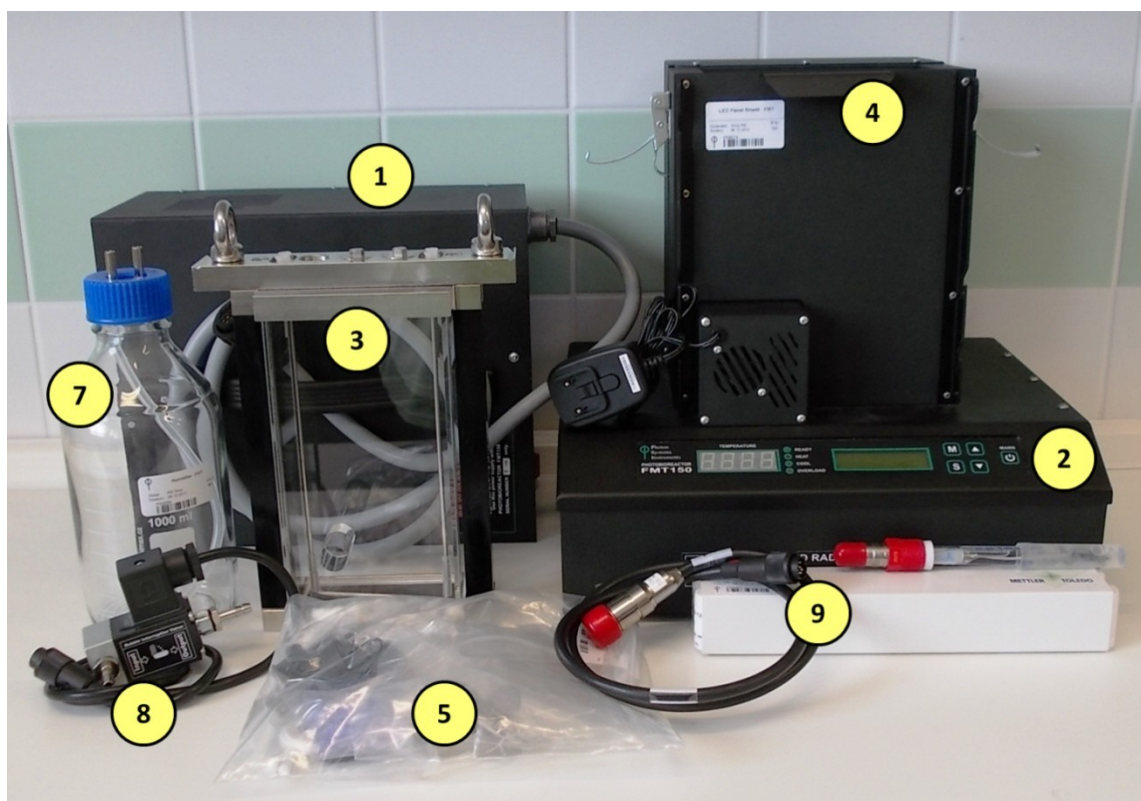


Fig. 5: Minimal configuration set of the Photobioreactor FMT150. Numbers correspond to component numbers as described on page 12.

3.3 CONTROL UNIT FRONT PANEL

Photobioreactor Control Unit front panel can be used for simple manual control of the PBR FMT150. By using the keys on the front panel the operation of cultivation and monitoring units such as pH/temperature sensor, light control, OD monitoring module, PWM mode or CO₂ and O₂ sensor can be controlled. When FMT150 photobioreactor is purchased without the Remote Control System for PBR FMT150 then all of the control has to be set up manually using the front panel of the FMT150 control unit. The user can read out from the display the actual values for the given sensor or monitoring unit.

Important: Please note that no user-defined protocols can be designed and no data recording is possible without the PC and PBR software.

Note: Please note that neither turbidostat nor chemostat module can be operated if FMT150 is purchased without the PBR software.

The front panel of the FMT150 control unit has 4 LED lights on the left and 5 control keys on the right (Fig. 6).

3. Device Description

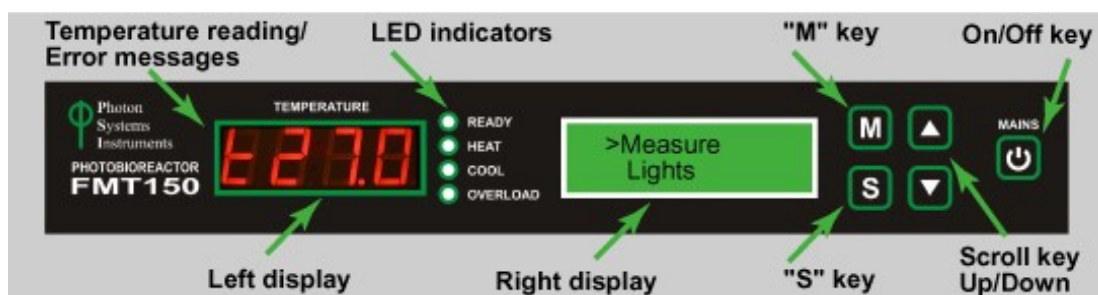


Fig. 6: Photobioreactor front panel

3.3.1 LED INDICATORS

Green LED indicator **READY** is lighting when the current temperature is equal to target temperature.

Orange LED indicator **HEAT** is lighting when the heater turns on in the cultivation vessel.

Blue LED indicator **COOL** is lighting when the water in the cultivation vessel is cooled and the Cooling Unit is operating. The minimal temperature in the standard PBR FMT150 is 10 °C.

Red LED indicator **OVERLOAD** is lighting when the requested temperature in the cultivation vessel higher than the current temperature was not reached yet.

3.3.2 DIGITAL DISPLAY ON THE LEFT

Displays the current temperature in the cultivation vessel and error messages if any problems with the FMT150 occur. Possible error messages are:

- **Er6** – Temperature/pH sensor is not connected.
- **Er7** - Temperature sensor is not functional and must be repaired. Please contact support@psi.cz if this error occurs.

3.3.3 DISPLAY ON THE RIGHT

This display shows various functions of the FMT150 and these are activated and set with the main keys on the right side of the control unit as described below.

3.3.4 MAIN KEYS

- **[M]:** Used to move back in the menu tree or to exit the menu.
- **[S]:** Used to move forward in the menu tree, to save the selection, or to turn ON/OFF.
- **[▲]:** Used to move up in the menu or to add value.
- **[▼]:** Used to move down in the menu or to subtract value.
- **[Mains]:** (On/Off) key doesn't have any function related to the common use of PBR and it is intended only for service purposes. The Photobioreactor is turned on by switching on the corresponding Power supply.

Note: Please refer to the chapter 8.1 if Photobioreactor FMT150 is purchased without the PBR software and the PBR FMT150 is to be controlled solely manually via the control unit front panel.

3. Device Description

3.4 REAR PANEL FOR VERSION FMT150.1

The rear panel houses connectors for all connecting cables (Fig. 7).

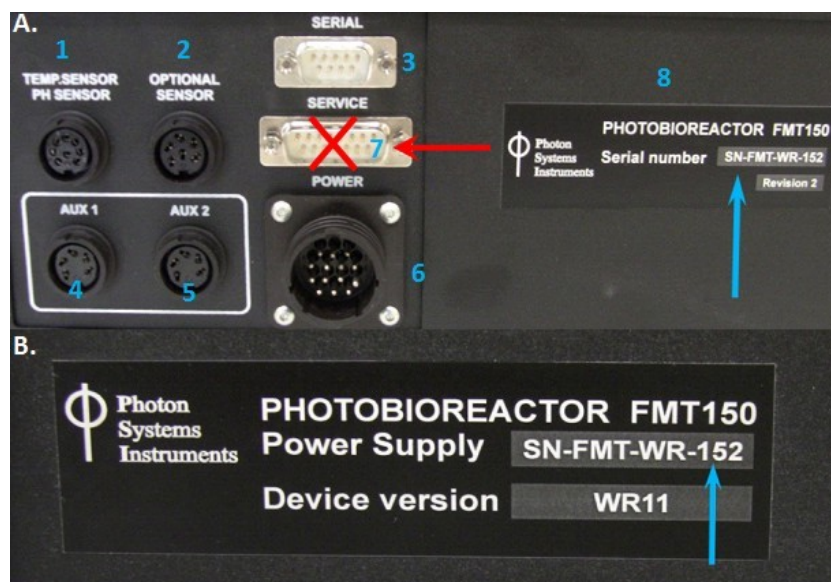


Fig. 7: Photobioreactor control unit and its power supply rear panels. A) Back panel of the Photobioreactor control unit with the serial number (blue arrow). Numbers correspond to the description below. B) Rear panel of the power supply with the serial number (blue arrow). Note the same serial number. Each Photobioreactor has its own power supply and it cannot be interchanged with another power supply.

3.4.1 CONNECTOR FOR PH/TEMPERATURE SENSOR

pH/temperature sensor should be plugged to this connector when in use. PBR FMT150 control unit recognizes when the sensor is connected and will automatically control the operation of the sensor.

3.4.2 CONNECTOR FOR OPTIONAL SENSOR

O₂ sensor should be plugged to this connector when in use. PBR FMT150 control unit recognizes when the sensor is connected and will automatically control the operation of the sensor.

Note: Only O₂ sensor can be plugged to this connector.

3.4.3 SERIAL COMMUNICATION CONNECTOR

USB connector with serial communication cable is provided as part of the PBR FMT150 package when purchased with the PBR software to connect the PBR FMT150 with the computer. This connection has to be made for the communication of the PBR FMT150 via the PBR software.

3.4.4 AUX1 CONNECTOR

AUX1 connector should be used for the CO₂ sensor, peristaltic pump, additional light or magnetic stirrer (please see page 20 for detail description of possible connection schemes).

3. Device Description

3.4.5 AUX2 CONNECTOR

AUX2 connector is used primarily for the bubble interruption valve, but can be used also for CO₂ sensor, peristaltic pump, additional light or PWM pump (please see Fig. 9 page 20 for detail description of possible connection schemes).

3.4.6 POWER SUPPLY CONNECTOR

The power supply unit is plugged into the FMT150 control unit via this connector.

3.4.7 SERVICE COMMUNICATION CONNECTOR

Service communication connector is designed only for servicing the FMT150 by the manufacturer.

Warning: NEVER CONNECT THE SERVICE CABLE TO THIS CONNECTOR (RED ARROW IN Fig. 7A) WITHOUT PRIOR CONSULTATION WITH THE MANUFACTURER.

3.4.8 IDENTIFICATION LABEL WITH SERIAL NUMBER

Each Photobioreactor FMT150 produced has a serial number that corresponds with the serial number of the power supply unit. These are always provided together and are not interchangeable. Prior to switching PBR FMT150 device ON make sure that the serial number on the rear panel of the main body matches the serial number on the power supply (Fig. 7B).

Warning: Damage can occur when the power supply is incompatible with the PBR FMT150 device.

3.5 REAR PANEL FOR VERSION FMT150.2

The rear panel houses connectors for all connecting cables (Fig. 8). Please note that the numbers in the next section correspond to the numbers in Fig. 8.

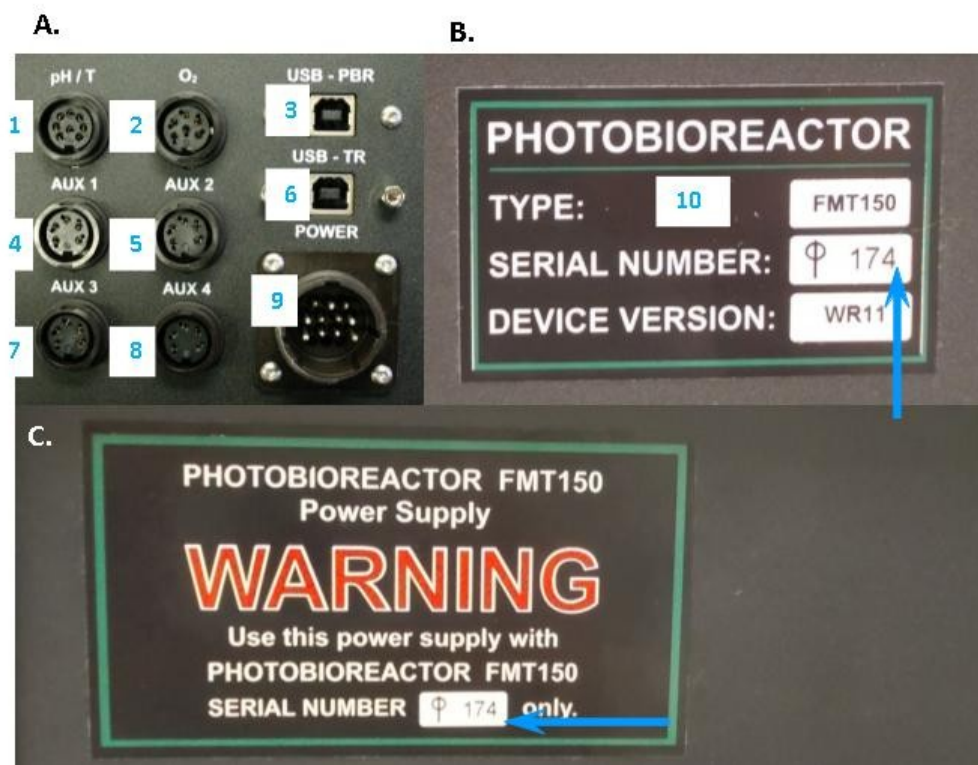


Fig. 8: Photobioreactor control unit and its power supply rear panel. A) Back panel of the Photobioreactor control unit. B) Serial number on the front panel of the of the PBR FMT150 unit. C) Rear panel of the power supply with the serial number (blue arrow).

3.5.1 CONNECTOR FOR PH/TEMPERATURE SENSOR

pH/temperature sensor should be plugged to this connector when in use. PBR FMT150 control unit recognizes when the sensor is connected and will automatically control the operation of the sensor.

3.5.2 CONNECTOR FOR O₂ SENSOR

O₂ sensor should be plugged to this connector when in use. PBR FMT150 control unit recognizes when the sensor is connected and will automatically control the operation of the sensor.

3.5.3 USB PBR COMMUNICATION CONNECTOR

USB communication cable is provided as part of the PBR FMT150 package when purchased with the Remote Control System to connect the PBR FMT150 with the computer. This connection has to be made for the communication of the PBR FMT150 via the PBR software.

3.5.4 AUX1 CONNECTOR

AUX1 connector should be used for the CO₂ sensor, peristaltic pump, PWM pump, additional light or magnetic stirrer (please see Fig. 9 on page 20 for detail description of possible connection schemes).

3. Device Description

3.5.5 AUX2 CONNECTOR

AUX2 connector should be used primarily for the bubble interruption valve but can be also used for CO₂ sensor, peristaltic pump, additional light (please see see Fig. 9 on page 20 for detail description of possible connection schemes).

3.5.6 USB TR COMMUNICATION CONNECTOR

USB TR communication connector is designed only for servicing the FMT150 thermoregulator by the manufacturer.

Note: NEVER USE THE USB TR CONNECTOR WITHOUT PRIOR CONSULTATION WITH THE MANUFACTURER.

3.5.7 AUX3 AND AUX4 CONNECTORS

AUX3 and AUX4 connector are additional connectors that can be used for the CO₂ sensor, peristaltic pump, additional light.

3.5.8 POWER SUPPLY CONNECTOR

The power supply unit is plugged into the FMT150 control unit via this connector.

3.5.9 IDENTIFICATION LABEL WITH SERIAL NUMBER

Each Photobioreactor FMT150 has a serial number that corresponds with the serial number of the power supply unit. These are always provided together and are not interchangeable. Prior to switching PBR FMT150 device ON make sure that the serial number on the front panel of the main body matches the serial number on the power supply (Fig. 8C).

Warning: Damage can occur when the power supply is incompatible with the PBR FMT150 device.

3.6 CONNECTION SCHEMA

Depending on the use of optional modules and the set-up of the PBR FMT150, the connection schema for the optional and standard modules with the PBR FMT150 control unit rear panel may differ. Please refer to Table 4 below to see the possible order of connecting the different modules to the PBR FMT rear panel.

Devices for which the interconnection is possible can be connected together in a series by AUX IN and AUX OUT connectors according to the recommended order. Please refer to Fig. 9 for recommended schematic connection setup for all available modules and accessories.

DEVICE USED	PROPER CONNECTOR	DEVICE INTERCONNECTION POSSIBLE
Peristaltic Pump	AUX 1/2/3/4	YES
CO ₂ module	AUX 1/2/3/4	YES
External Light	AUX 1/2/3/4	YES
Bubble Interruption Valve	AUX 2	NO
Magnetic Stirrer	AUX 1	NO
PWM Pump	AUX 1	NO

Table 4: External connectors

Important: Please note that Bubble Interruption Valve must be always connected to the AUX2 connector and Magnetic stirrer and PWM pump always to connector AUX1. In case of magnetic stirrer and PWM Pump, only one of the two devices can be connected to the PBR FMT150 at one time.

Note: Please note that the AUX3 and AUX4 connectors are available only in the FMT150.2.

3.Device Description

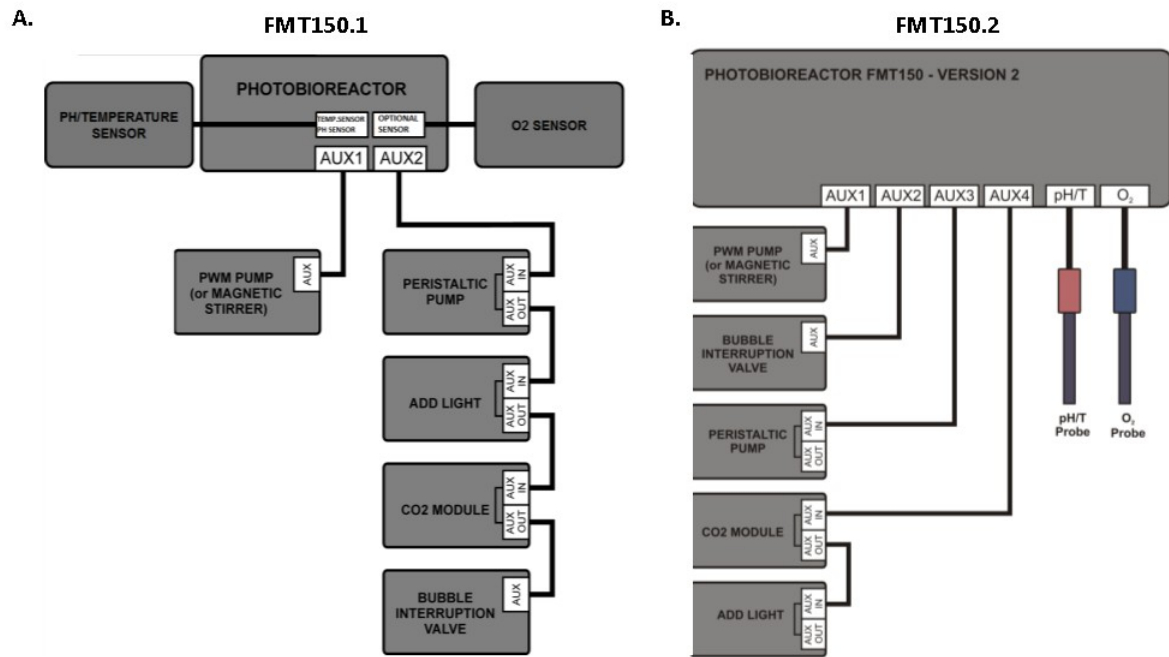


Fig. 9: Schematic of recommended set-up for interconnecting available modules with PBR FMT150 control unit. A) Scheme valid for FMT150.1 version. B) Scheme valid for FMT150.2 version.

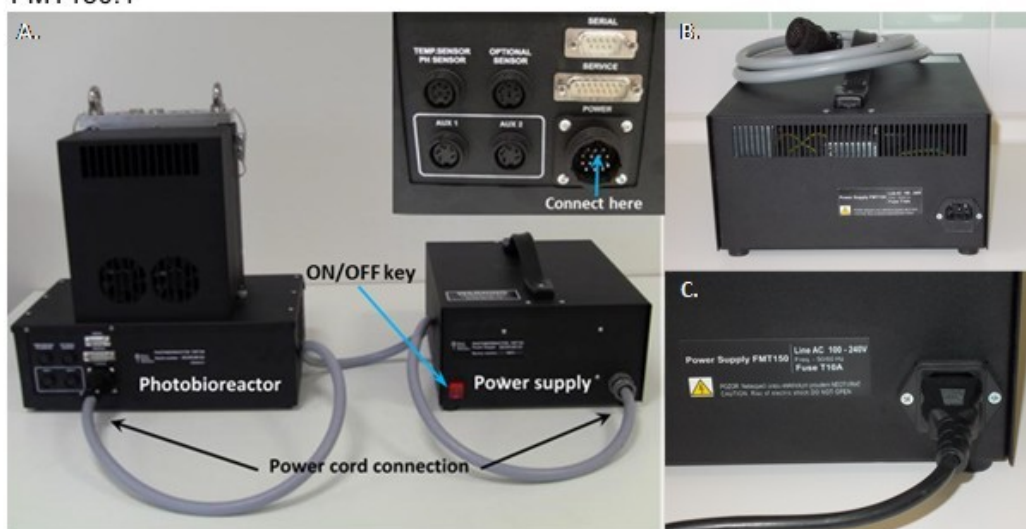
4. DEVICE INSTALLATION

To install the device properly, it is necessary to follow the proper sequence of the assembly instructions. Instructions for assembly of standard PBR FMT150 (400, 1000 and 3000 ml) are described below.

4.1 POWER SUPPLY

- Make sure that both the Photobioreactor and the power supply are switched OFF.
- Connect the thick power cord of the power supply to the round Power connector at the rear panel of the Photobioreactor (Fig. 10).

FMT150.1



FMT150.2

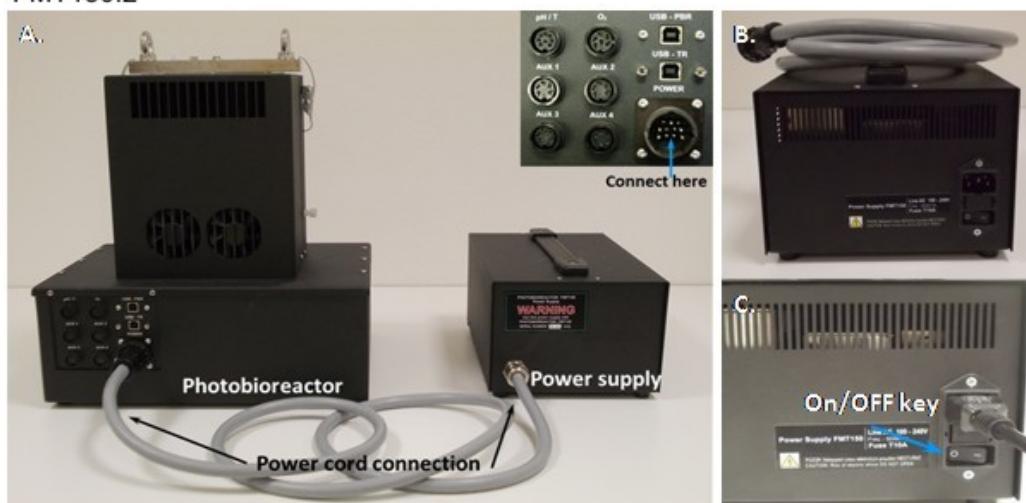


Fig. 10: Connection of the Photobioreactor and the power supply. A) Top right panel shows detail view of the back panel of the Photobioreactor. B, C) AC power cord connection to the electrical outlet.

- Plug the AC power cord into an electrical outlet (Fig. 10B,C)
- Switch on the power supply

4. Device Installation

- **Important:** It is necessary to allow 20 minutes stabilisation time for the device prior any experiment or calibration is performed. This stabilisation time is required for temperature stabilisation of the measuring system.

Important: It is strongly recommended to use back-up UPS system to protect the PBR FMT150 system from **power outage**. If UPS system is not used and the AC power is turned off unexpectedly the PBR FMT150.1 control unit needs to be switched ON with the MAINS key. After switching the unit ON, all previous settings will be reset. Please note that if PBR software is used and the power outage occurred all previous settings will be restored and the currently running experiments will continue.

In case of the PBR FMT150.2, control unit will automatically switch ON after the AC power is restored again and no manual action is required.

4.2 CULTIVATION VESSEL

The standard cultivation vessel has capacity of 400 ml, 1000 ml or 3000 ml (depending on the unit ordered). It is made from flame-proof glass (thickness 3.3 mm), stainless steel (AISI316L), duralumin with black eloxal coating, silicone sealing, and Lukopren S6410T glue. Each vessel is tested and autoclaved (20 minutes at 120 °C) by the manufacturer before distribution.

VOLUME	WEIGHT	DIMENSIONS
400 ml	2,8 kg	23,88 x 3,70 x 19,3 cm
1000 ml	3,8 kg	23,88 x 7,36 x 19,3 cm
3000 ml	6,2 kg	31,70 x 7,36 x 30,3 cm

Table 5: Weights and dimensions

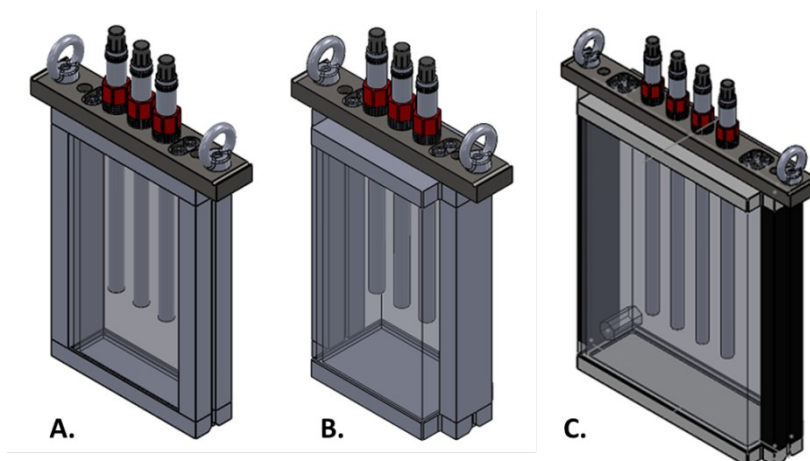


Fig. 11: Schematic design of the PBR cultivation vessel types. A) Vessel for PBR FMT 150/400. B) Vessel for PBR FMT 150/1000. C) Vessel for PBR FMT 150/3000.

4. Device Installation

4.2.1 SETUP

The lid of the vessel is fastened to the vessel via two screws (n°3 in Fig. 12C) using the hex key provided. The lid has two holders built in for easy handling of the vessel in and out of the photobioreactor (n°2 Fig. 12C). There are four openings for luer connectors. These are used for connection of aeration tubing, sampling tubing and waste expel tubing (Fig. 12B). The lid also has three larger openings for optional sensors such as pH/Temperature, O₂, CO₂ probes. If sensors are not used these openings can be kept closed with the filler screws provided.

The stainless steel aeration tubing mounted to the bottom of the vessel lid is connected via two Luer Lock connectors with the gas dispenser tubing (n°4 in Fig. 12A) linked via a T-piece to the humidifier bottle (Fig. 12B). The source gas for the culture (gas pump or gas mixing system) is humidified before entering the culture vessel and this helps to maintain a constant volume in the vessel. The third luer lock connector is linked with silicone tubing (n°7 in Fig. 12A,C), which serves for removal of residual or overflow medium.

Note: The longer metal thin tubing on the vessel lid is used for medium outlet.

The fourth luer outlet can be either closed by a Lock Ring Plug (component n°4 in Table 3) or connected with silicone tubing and used as a sampling outlet or for medium in-flow when connected with peristaltic pump (n°6 in Fig. 12A,C).

Note: Sampling tube can be used as gas outlet when Gas Analysis System (GAS) is connected to the PBR FMT150; for more information see page 46.

Once the cultivation vessel is set up and placed in the photobioreactor it should be locked to the body of the PBR FMT150 by the clips on each side of the PBR

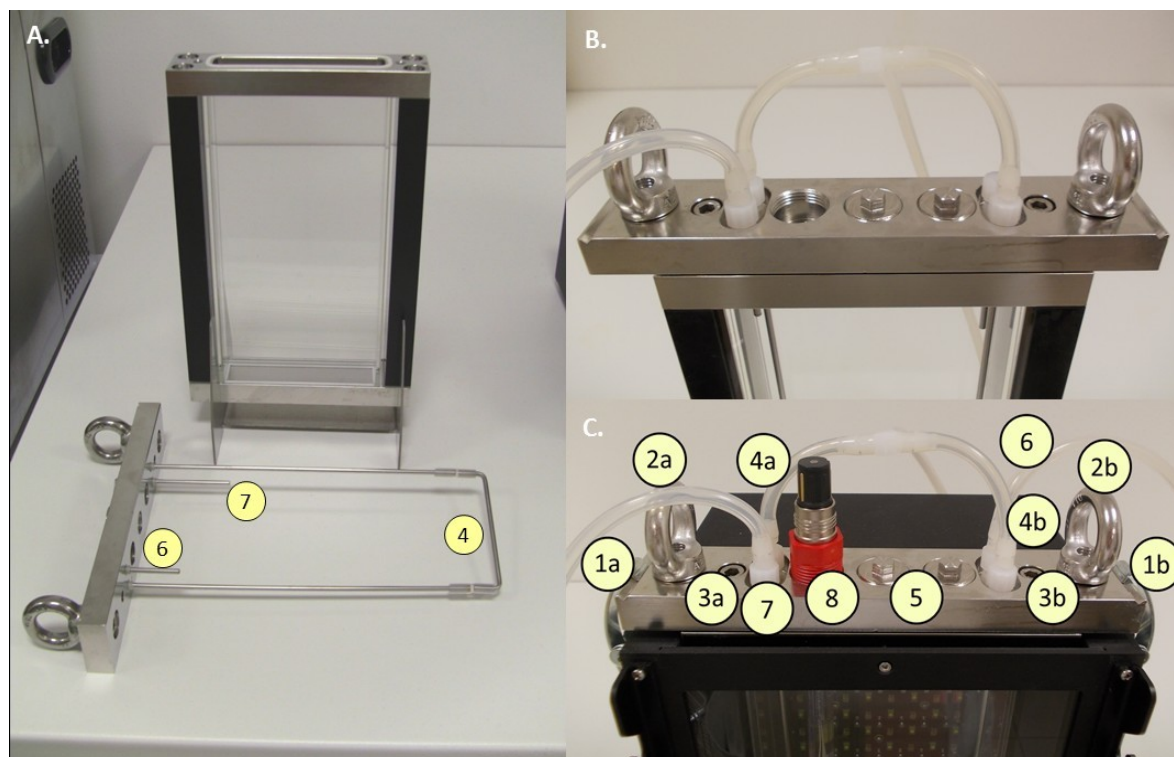


Fig. 12: Components of the cultivation vessel 400ml. A) vessel and the vessel lid; numbers correspond to description in C. B) Top of the lid with connection of the aeration tubing. C) Vessel lid with one sensor and all tubing connections: (1a,b) Clips to lock the vessel to the photobioreactor. (2a,b) Holders for easy vessel manipulation. (3a,b) Screws for fastening part of the lid to the vessel. (4a,b) Inlets for the gas dispenser tubing. (5) Connectors for optional sensors closed with filler screws. (6) Sampling tube. (7) Excess medium outlet. (8) pH/temperature sensor.

4. Device Installation

Important: Please note that if the vessel is removed in 1000 ml or 3000 ml version of the PBR FMT150, calibration constants stored in the system after OD calibration will no longer correspond to the new conditions. In 1000 ml or 3000 ml PBR FMT150 version glass tunnel is used to improve optical path for OD and fluorescence measurements, therefore any shift in the position in the vessel might slightly change the optical path of the measuring beam and influence the absolute measured values. Please refer to the OD calibration procedure for detail information.

4.2.2 MAINTENANCE

The whole vessel is fully autoclavable, including the detachable lid, connectors, sensors, and silicone tubing. However, keep in mind that most materials deteriorate after repeated autoclaving. We recommend that you keep autoclaving to a reasonable minimum. For most cases, hot water (60 – 70 °C), standard detergent and thorough mechanical cleaning are enough to keep the equipment clean and non-contaminated. Optionally, the vessel and all the tubings can be disinfected with 70% ethanol. When cleaning the cuvette, it is very important to rinse off any residue of detergent or medium and to dry the cuvette with a soft cloth – this will help to prevent the cuvette corrosion. The cuvette should not come in contact with strong acids or acid vapours.

The O₂ sensor and pH sensor may be autoclaved or sterilized up to 140 °C; the CO₂ sensor may be autoclaved or sterilized up to 130 °C.

4.2.3 ASSEMBLY

Note: Please refer to chapter 4.2.4 for details about description and of 3000 ml vessel.

The next instructions describes cultivation vessel assembly:

- Remove the lid from the vessel by loosening the two screws with the provided hex key.
- Fill the vessel with medium, place the stir bar inside the vessel if used with the magnetic stirrer.
- Prepare the gas dispenser tubing by connecting the T-piece (n°16 in Table 3) with longer silicone tubing. Connect the gas dispenser tubing to the two luer connectors as shown in Fig. 13A,B.

Note: Standard version of the PBR FMT150 is connected according to the Fig. 12B, when optional modules such as peristaltic pump or GAS and GMS are used see page 43 and 46 for further instructions).

- Close the last Luer Lock connector with the Luer Lock Ring Plug (n°4 in Table 3).
- Re-attach the lid to the vessel (Fig. 12C-3) using provided hex key. Make sure that the rubber gasket between the vessel and the lid is properly installed and a good seal is achieved.

If needed, autoclave the assembled vessel.

Important: Never close all three sensor holes with the screws during autoclaving. It is recommended to leave one sensor hole empty and cover the lid completely with aluminium foil to prevent pressure build-up during autoclaving and keep the vessel protected from any contamination prior experiment initiation. Provided sensors such as pH/temp, O₂ and CO₂ probes can be autoclaved but their lifetime is reduced by repeated autoclaving.

- Insert the assembled and prepared vessel into the Photobioreactor and lock it using the metal clips (Fig. 12C-1).

Note: For detail description of recommended protocol for initiation of cultivation for algae and cyanobacteria in PBR FMT150 please refer to chapter 6 on page 56.

4. Device Installation

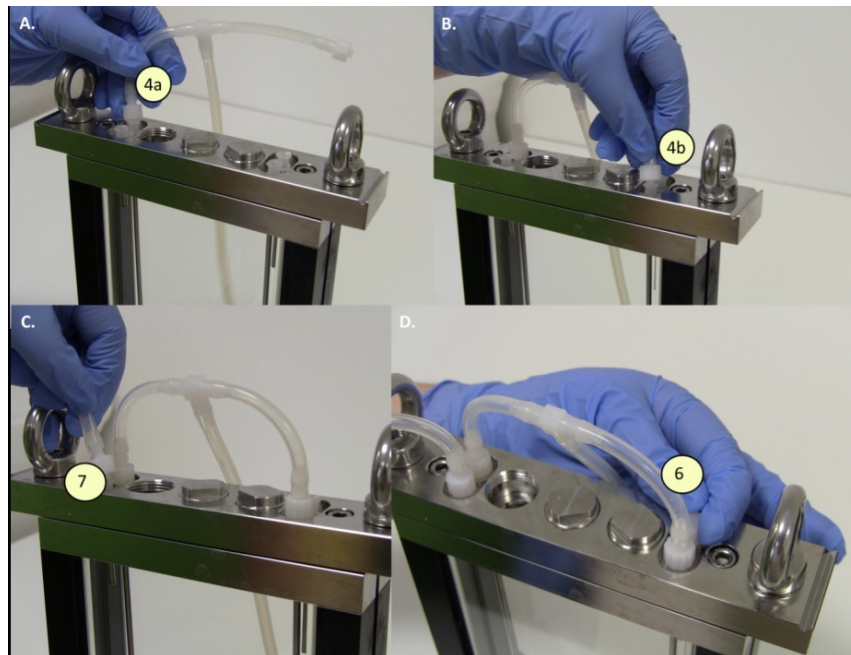


Fig. 13: Assembly of the vessel lid. A-D) Individual steps of standard vessel assembly set-up are shown. Please note that the numbers in yellow bullets correspond to numbers in Fig. 21C.

4.2.4 3000 ML VESSEL SPECIFICS

The PBR FMT150/3000 provides more connectors than in 400/1000ml models to give the user broader range of connected optional instruments and larger scope of operational features.

Please refer to Fig. 14 for detail description of the 3000ml vessel lid and all the connectors:

- (2) Holders for easy vessel manipulation.
- (3) Screws for fastening part of the lid to the vessel.
- (4) Inlets for the gas dispenser tubing.
- (5) Connectors for optional sensors closed with filler screws.
- (6) Sampling tube for turbidostat mode. Inlet is used to add medium to the vessel or to collect the samples.
- (7) Excess medium outlet.
- (9) Inlets for chemostat mode. Inlets for base and acid chemostat mode.
- (10) Optional inlets (e.g. for multiple peristaltic pump connection).

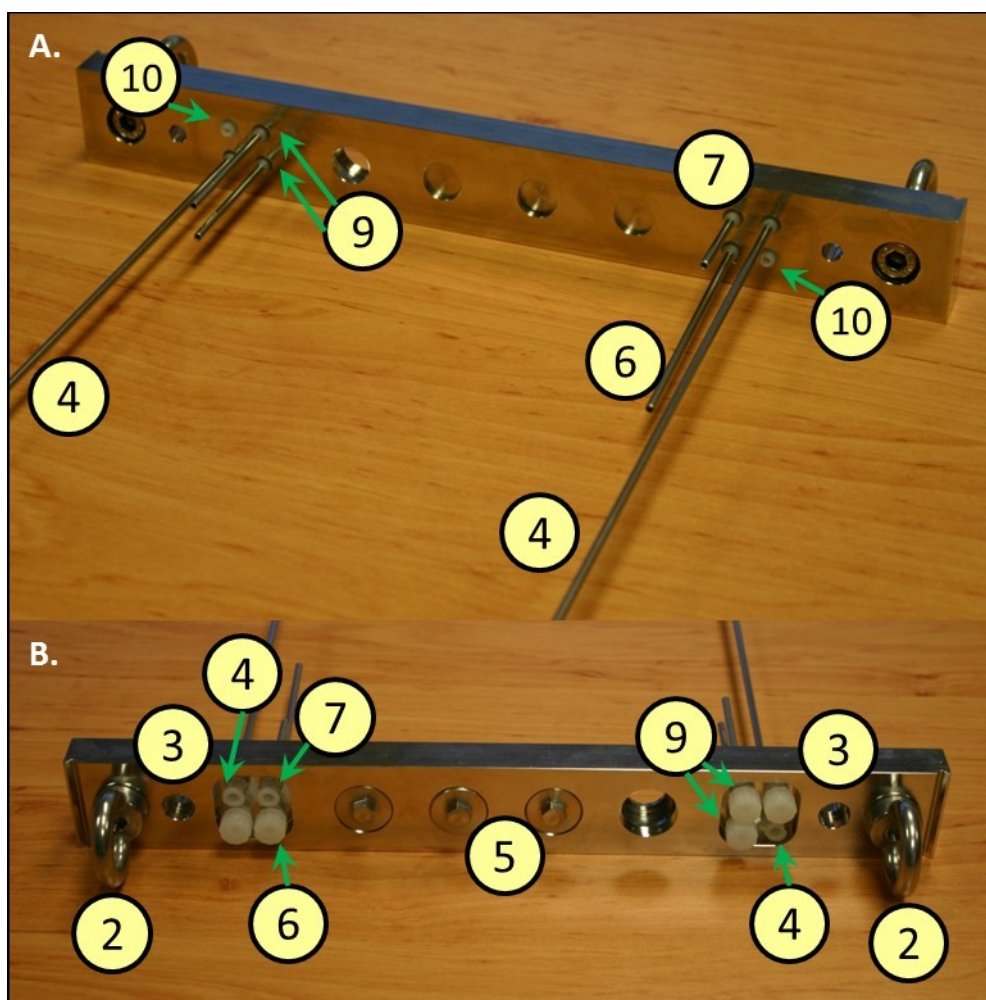


Fig. 14: Components of 3000 ml cultivation vessel

4.3 PH/TEMPERATURE SENSOR

The pH/temperature module is designed for continuous measuring of pH and temperature in the medium. pH/temperature sensor is part of minimal required configuration (described in chapter 3.2) and is recommended for purchase if precise control of cultivation conditions is required.

The module consists of electronic unit embedded in Photobioreactor FMT150 Control Unit, probe and communication cable (Fig. 20).

IMPORTANT NOTES: It is strongly recommended to avoid autoclaving pH electrode. The manufacturer states that autoclaving deteriorates it rapidly. Use warm water with a detergent or chemical disinfectants for its cleaning.

4.3.1 INSTALLATION

- Open one of the large ports in the lid by unscrewing one of the steel screws in the lid.
- Insert the pH electrode into the open port and secure it tightly with a plastic ring around the probe (Fig. 15A,B).

Note: Any position in the lid can be selected for the pH/Temperature sensor. However, we recommend placing the O₂ sensor in the far right connector of the vessel, where magnetic stirrer provides most homogenous mixing of the media.

4. Device Installation

- Attach the cable labelled “pH” to the pH/Temperature electrode (Fig. 15C).
- Connect the connector cable with the PBR FMT150 (Fig. 15D).
- When connected to the PBR FMT150 control unit, sensor unit is automatically recognized. The function of the pH/temperature sensor can be controlled manually by the front panel of the control unit and remotely via PBR software. For manual control please refer to control menu tree on page 59 for more detail explanation.

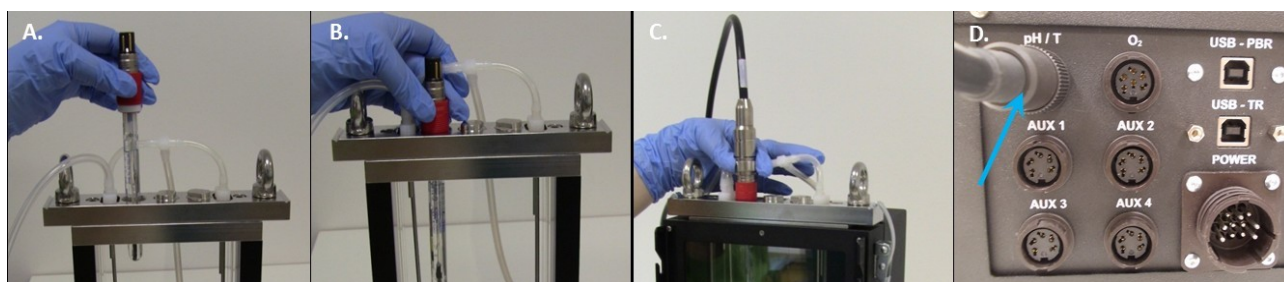


Fig. 15: Installation of the pH/temperature sensor. A) Positioning the probe. B) Securing the probe. C) Attaching the cable to the positioned probe. D) Attaching the cable to the rear side of the PBR.

4.3.2 PH CALIBRATION

Prior to initiation of each experiment it is recommended to perform the pH sensor calibration. The calibration can be done either in PBR software (for more details see PBR Software manual) or using the FMT150 control unit front panel as described below.

Follow these instructions during calibration:

- Go to operation menu using the front panel of the PBR control unit. In the operation menu go to Setting → Calibration → Calibration pH.

IMPORTANT Note: If you have older versions of the electrodes InPro3253/120/PT1000 you need to connect the device to earth during the process of calibration. For the new version of the electrode: InPro3253SG/120/PT1000 calibration can be done without grounding. See Fig. 16 C and D for description of the grounding (For InPro3253/120/PT1000 version of the electrode, place one end of a copper wire in the calibration solution and the other end fix under the metal clip on the PBR main body as shown in Fig. 16 C, D. Proceed with the calibration).

- Place the pH sensor connected to the PBR in pH = 7 buffer calibration solution (Fig. 16 A)

Note: Please note that the calibration solutions are not provided by the manufacturer.

- Read the signal value on the display of the photobioreactor control unit (Fig. 16 B), wait until it is stable and confirm this value by pressing the “S” key.
- Repeat the same procedure with buffer solution of pH = 10.
- When calibration is done, press the “M” key to get back to the main menu.
- **Optional:** Calibration accuracy may be verified by plunging the electrode in the same calibration solutions of known pH (pH value in between 7 and 10).

4. Device Installation

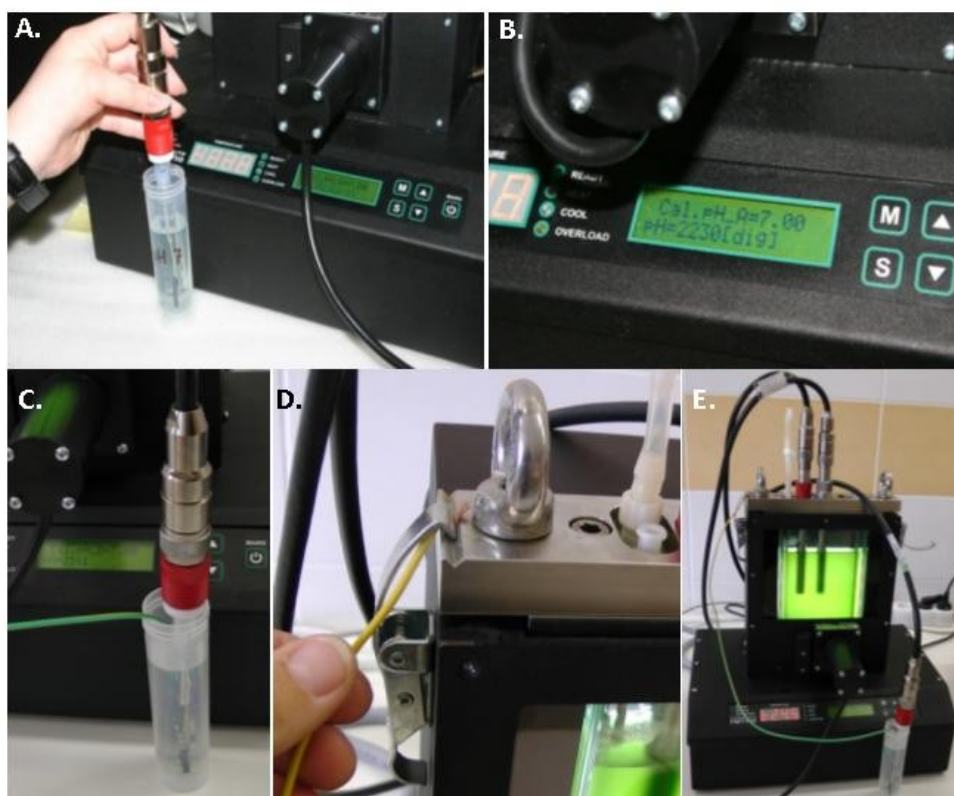


Fig. 16: pH sensor calibration. A) Electrode is placed into the solution of pH 7. B) Reading of the measured value. C, D, E) Grounding with the copper cable for the calibration of InPro3253/120/PT1000 version of the electrode.

4.4 GAS SUPPLY

The gas supply to the photobioreactor consists of four main parts – pressurized gas source, bubble humidifier, the bubble interruption control valve, and gas dispenser tube (Fig. 18). All parts are connected with flexible silicone tubing.

The following instructions describe the proper installation of the gas supply for the photobioreactor:

- Connect gas source (**Note:** this can be pressurized gas from air pump or pressurized mixed air from other sources) with the bubble interruption valve via the silicone tubing.
- Prepare the bubble humidifier bottle: Components needed for bottle assembly are shown in Fig. 17A. Connect silicon tubing to the metal portion of the lid with the ports and insert into the screw cap. One end of the silicon tubing has FTLL Luer Lock (n°3 in Table 3) and Lock Ring Plug (n°4 in Table 3), the second has MTLL Luer Lock (n°1 in Table 3), as shown in Fig. 17B. Other silicon tubing of about 15-20 cm is connected to the other side of the metal sleeve (Fig. 17B). Fill the bottle up to 1 litre with distilled water, insert the sealing ring into the screw cap and close loosely the bottle with the assembled lid and cover by aluminium foil. The ends of all the tubings should be covered with aluminium foil and the assembled aeration bottle should be autoclaved (Fig. 17C).
- After autoclaving, connect the bubble humidifier bottle as described in the following steps. Remove the Lock ring plug from the silicon tubing and connect the MTLL Luer Lock with FTLL Luer Lock placed in the main gas dispenser tubing. Prepare silicon tubing of approximately 30-35 cm length and connect it with bubble

4.Device Installation

interruption valve as shown in Fig. 18. Connect 20 μm air filter (**Note:** air filter is not part of the components provided with PBR FMT150) with the MTLL Luer Lock end of the silicon tubing from the humidifier bottle and via other FTLL Luer Lock connect with the silicon tubing coming from the bubble interruption valve.

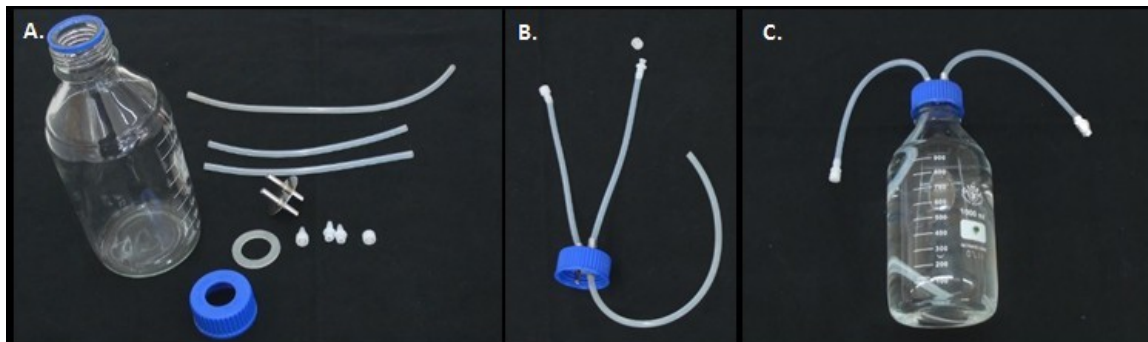


Fig. 17: Assembly of the bubble humidifier bottle. A) Components needed for the assembly of the bottle. B) Assembled lid. C) Assembled bubble humidifier bottle.

- Connect the bubble interruption valve cable with the AUX2 connector on the back of the FMT150 or with the peristaltic pump via the AUX Out (see Fig. 9 on page 20)
- Switch on the air pump or other pressurized gas source.

Note: Make sure that the waste fluid outlet tubing from the PBR is placed inside of the waste bottle. The gas from the PBR escapes via this tubing.

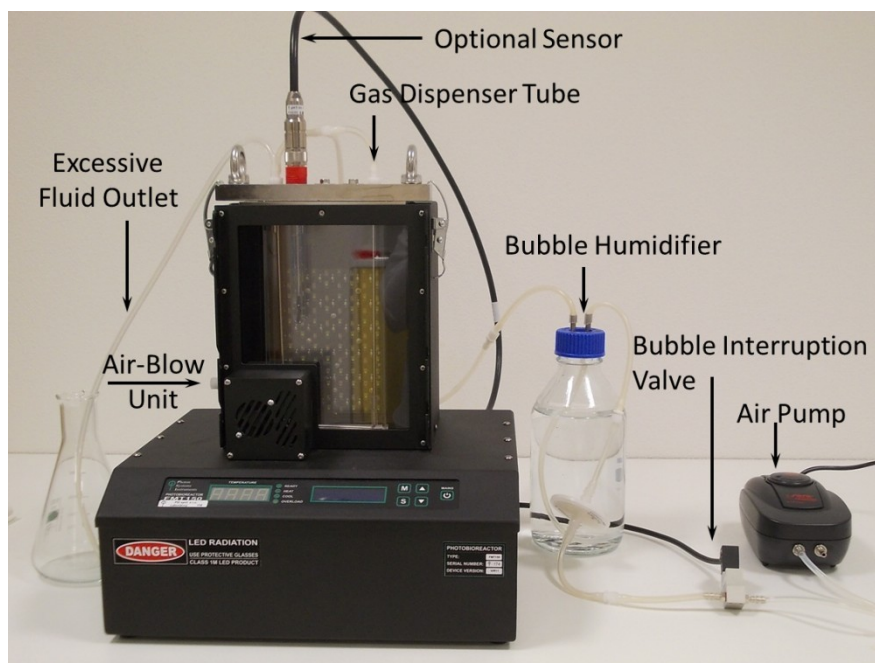


Fig. 18: FMT150 set-up with air pump supplying the gas.

5. OPTIONAL ACCESSORIES/COMPONENTS

The functions of the PBR FMT150 may be enhanced by wide range of optional components. Installation and operation of the optional accessories that can be provided together with the PBR FMT150 device are described in next chapters.

5.1 PBR SOFTWARE

The Photobioreactor can be connected with an external PC that runs PBR software (Fig. 19). The PBR software can define, control, visualize and analyze experimental data and settings (overriding the photobioreactor controller) like

- duration of the experiment
- set up of user-defined protocols
- sensors data recording in real time
- regulation of temperature dynamics, LED irradiance, stirring, pumps, gas mixer, ...
- data export in various formats (Excel, CSV, XML)
- data visualization in graphs
- remote control via internet

The PBR software is necessary for recording of measured parameters from both the photobioreactor and the auxiliary sensors. If the PBR computer is connected to the internet, the data display, as well as regulation of the growth conditions can be managed remotely from anywhere.

The software operates on Linux operating system and allows. USB cable for PC and PBR FMT150 communication is supplied with the system (Please note that in older versions of FMT150 serial cable with USB adapter is included instead).

See the PBR Software manual for detailed description of software capabilities.



Fig. 19: Remote Control System for PBR FMT150. A) Version FMT 150.1; B) Version FMT 150.2.

5.2 AIR PUMP WITH BUBBLE INTERRUPTION VALVE

Air pump for culture bubble stirring and supply of air levels of CO₂. Valve interrupts bubbling during measurements, it is required for precise readings of OD, Ft and QY. Bubble interruption valve can be purchased also without air pump if required. The component is shown on Fig. 5 on page 14 under number 8.

5.3 PH/TEMPERATURE MODULE

To measure pH and temperature. It is required for cultivation thermoregulation. The module consists of electronic unit embedded in Photobioreactor FMT150 Control Unit, sensor probe and probe cable (Fig. 20). **InPro3253SG/120/PT1000** or **InPro3253/120/PT1000** single rod measuring cells from METTLER TOLEDO (www.mt.com) are supplied as low maintenance, autoclavable and pressure resistant pH electrodes with a factory filled pre-pressurised liquid electrolyte and a reference electrolyte together with a silver ions barrier. Measurement disturbances caused by earth potentials are avoided through employment of a “solution ground” option available with InPro3250SG version. For InPro3250 version please refer to description of grounding during calibration on page 27. For more information please refer to manual provided by electrode manufacturer.



Fig. 20: pH/temperature module.

5.4 O₂ MODULE

The O₂ module is designed for continuous monitoring of dissolved O₂ concentration in the culture medium. The module consists of electronic unit embedded in Photobioreactor FMT150 Control Unit, sensor probe and sensor communication cable (Fig. 21). O₂ sensor **InPro6800/12/120** from METTLER TOLEDO (www.mt.com) with integrated temperature probe is used for oxygen measurement. Operation of the **InPro6800/12/120** sensor is based on polarographic measurement. For more information please refer to manual provided by electrode manufacturer.



Fig. 21: O₂ Module

IMPORTANT NOTES:

- O₂ electrode is made of high-quality stainless steel, grade 316, which is autoclavable. Yet, we recommend to keep autoclaving to a reasonable minimum.
- Be aware that, although the O₂ electrode is made of high-quality stainless steel, a very aggressive growth medium can cause its corrosion. Consult your specific conditions with the electrode manufacturer.

5.4.1 INSTALLATION

Follow these instructions to install the O₂ sensor:

- Insert the O₂ electrode into the connector on the right side of the vessel lid and secure it tightly (Fig. 22A,B).
Note: The placement of the O₂ sensor on the right side of the vessel is recommended as it needs to be close to the magnetic stirrer to ensure homogenous mixing of the media and prevent localized depletion of O₂ near the electrode
- Attach the cable labeled “O₂” to the electrode (Fig. 22C)
- Attach this cable to the connector at the back panel of the Photobioreactor. See the blue arrow in Fig. 22D for FMT150.2 set up. In FMT150.1 attach it to “optional sensor” connector (see Fig. 7).

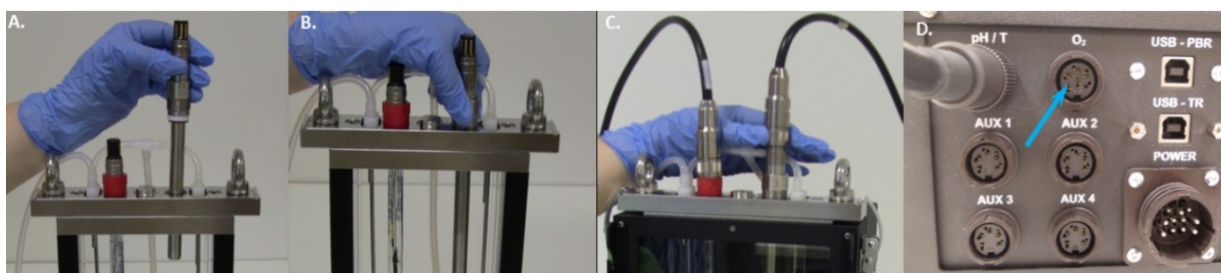


Fig. 22: Installation of the O₂ sensor. A) Positioning the electrode. B) Securing of the electrode. C) Attaching the cable to the positioned O₂ electrode. D) Attaching the cable to the rear side of the PBR FMT150.2.

- When connected to the PBR FMT150 control unit, the sensor is automatically recognized. The function of the O₂ sensor can be controlled manually by the front panel of the control unit or by PBR software. For manual control please refer to chapter 8.1 on page 59 for more detailed explanation.
- Before first use of the electrode we recommend to read the manual provided by the electrode manufacturer (www.mt.com).

5.4.2 CALIBRATION

The calibration of the dissolved O₂ probe can be performed with assistance of PBR software (for more details see the PBR Software manual).

Important: It is very important to calibrate the O₂ electrode at the temperature that will be used during experiment. The output of the O₂ electrode is not temperature compensated and it is sensitive to temperature. Follow the instructions provided by electrode manufacturer and description in the PBR Software manual to perform the calibration.

5.5 CO₂ MODULE

The CO₂ module is designed for continuous monitoring of dissolved carbon dioxide concentration in the medium. The module consists of external electronic unit for connection with Photobioreactor FMT150 Control Unit, sensor probe and sensor communication cable (Fig. 23).

InPro5000/12/120 sensor from METTLER TOLEDO (www.mt.com) with integrated temperature probe is used for potentiometric CO₂ concentration measurement. The InPro5000 sensor employs a gas permeable silicone membrane which is tightly stretched around a special engineered flat pH membrane. CO₂ partial pressure can be calculated using the pH and the temperature. For more information about CO₂ probe please refer to manual provided by electrode manufacturer.

5.Optional Accessories/Components



Fig. 23: CO₂ Module

5.5.1 INSTALLATION

Follow these instructions to install and operate the CO₂ sensor:

- Before the first use of the electrode we recommend reading the manual provided by the electrode manufacturer (www.mt.com).
- Assemble the electrode according to the manual provided by the electrode manufacturer. Individual steps of the assembly are schematically shown in Fig. 24.

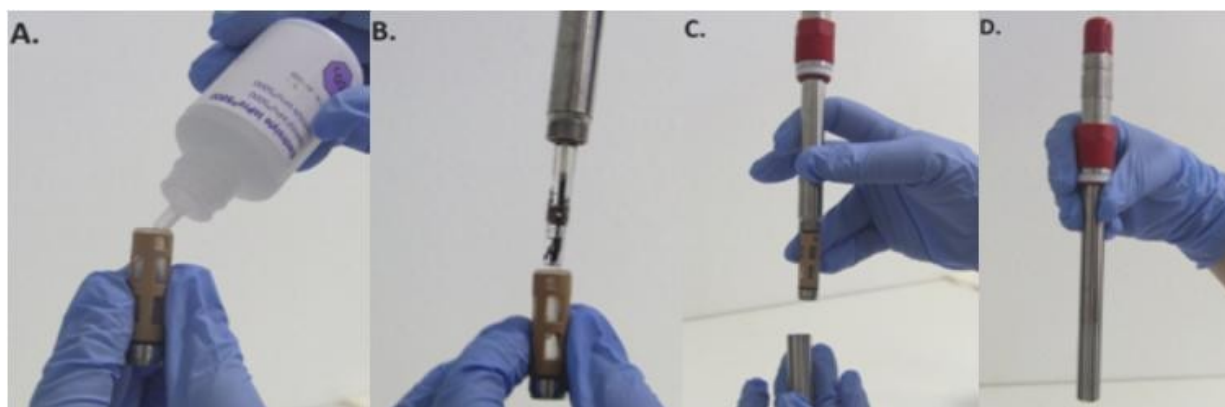


Fig. 24: Assembly of the CO₂ electrode

- Insert the CO₂ electrode into the appropriate vessel connector and secure it tightly (Fig. 25A).
- Note:** The position of the sensor in the lid is optional. In case other sensors are used, we recommend to place the O₂ sensor to the right side of the vessel, where magnetic stirrer assures more homogenous mixing of the media this will leave the central position available for the CO₂ probe.
- Attach the cable of the dissolved CO₂ module to the CO₂ electrode (Fig. 25B).
 - Connect the PBR FMT150 and the dissolved CO₂ module using a provided communication cable.
 - Plug the communication cable into the “AUX IN” connector of the electronic box of the dissolved CO₂ module (Fig. 25C).

5.Optional Accessories/Components

- Plug the other end of the communication cable into the “AUX1” connector at the rear of the Photobioreactor (Fig. 25D) or the AUX out of the additional components such as peristaltic pump as **inated** in Fig. 9 pg. 20 if other optional components are included.

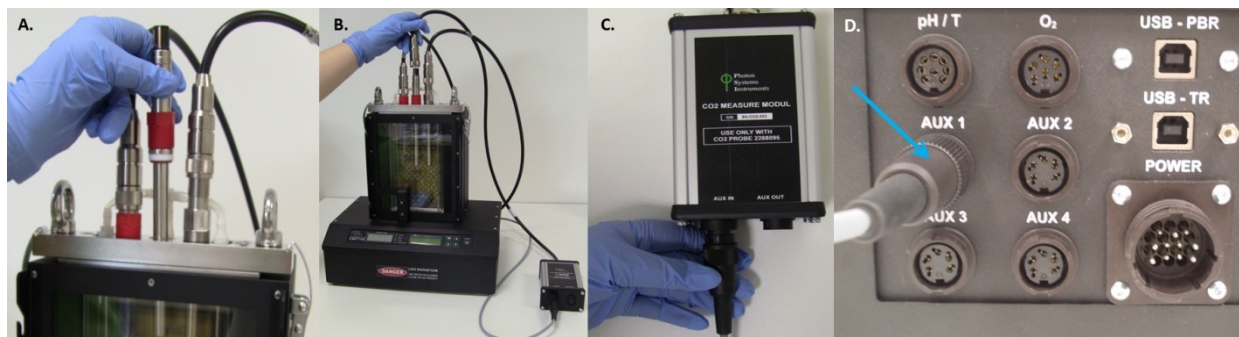


Fig. 25: Installation of the CO₂ sensor. A) Positioning the electrode. B) Attaching of the electronic module to the electrode. C) Attaching of the communication cable to the “AUX IN” connector of electronic module. D) Attaching of the communication cable to the “AUX 1” connector at the rear of the PBR.

- The function of the CO₂ sensor can be controlled manually by the front panel of the control unit or by PBR software. For manual control please refer to chapter 8.1 on page 59 for more detailed explanation.

5.5.2 CALIBRATION

To display CO₂ values as CO₂ concentration it is important to perform the calibration prior to the use of the electrode at the temperature of the cultivation conditions.

The calibration of the CO₂ probe can be performed with assistance of PBR software (for more details see PBR Software manual). Please follow also the instruction provided by the electrode manufacturer (www.mt.com) and instruction described below.

There are three different ways one can use to calibrate the CO₂ electrode for use in PBR FMT150:

1. Using the gas mixing system and pure CO₂ calibration gas where known CO₂ gas mixtures are prepared and then bubbled through the medium.
2. Using commercially prepared CO₂ gas mixtures in gas tanks, bubbling these into the medium.
3. Using standard concentration of calibration solution (NaHCO₃ and Na₂CO₃) and adding these to the medium.

Please note that in each case, the calibration procedure requires 3 concentration points and it takes about 12 hours. This is due to time required for each CO₂ concentration to equilibrate in the solution and the slow response rate of the electrode itself.

Below please find the guidelines for the CO₂ calibration based on use of standard calibration solutions (NaHCO₃ and Na₂CO₃). Briefly, given amount of NaHCO₃ and Na₂CO₃ is dissolved in deionized water to prepare standard calibration solution. A known volume of the standard calibration solution is added to the Photobioreactor vessel **completely** filled with deionized water that was aerated with N₂ to remove any residual CO₂. The solution in the vessel is stirred with the magnetic stirrer and the vessel is maintained closed during measurement so CO₂ gas does not escape. Example of calibration curve and table with concentration conversions factors is shown in Fig. 26.

Important: Please note that CO₂ electrode is designed for measurements of dissolved CO₂ in the range from 6×10^{-5} mol/L to 2×10^{-2} mol/L CO₂ which corresponds to the lower limit of detection around 60 μ M dCO₂ (2000 ppm in 25 °C). For measurements of dCO₂ close to ambient CO₂ concentration, it is recommended by the manufacturer to dilute

5.Optional Accessories/Components

supplied electrolyte solution 1:20 with distilled water and use it instead of the stock electrolyte solution. With 1:20 diluted electrolyte solution the response of the sensor is faster and more precise in the lower range of dCO_2 concentration. For more information about the electrolyte solution please refer to information provided by the electrode manufacturer (www.mt.com).

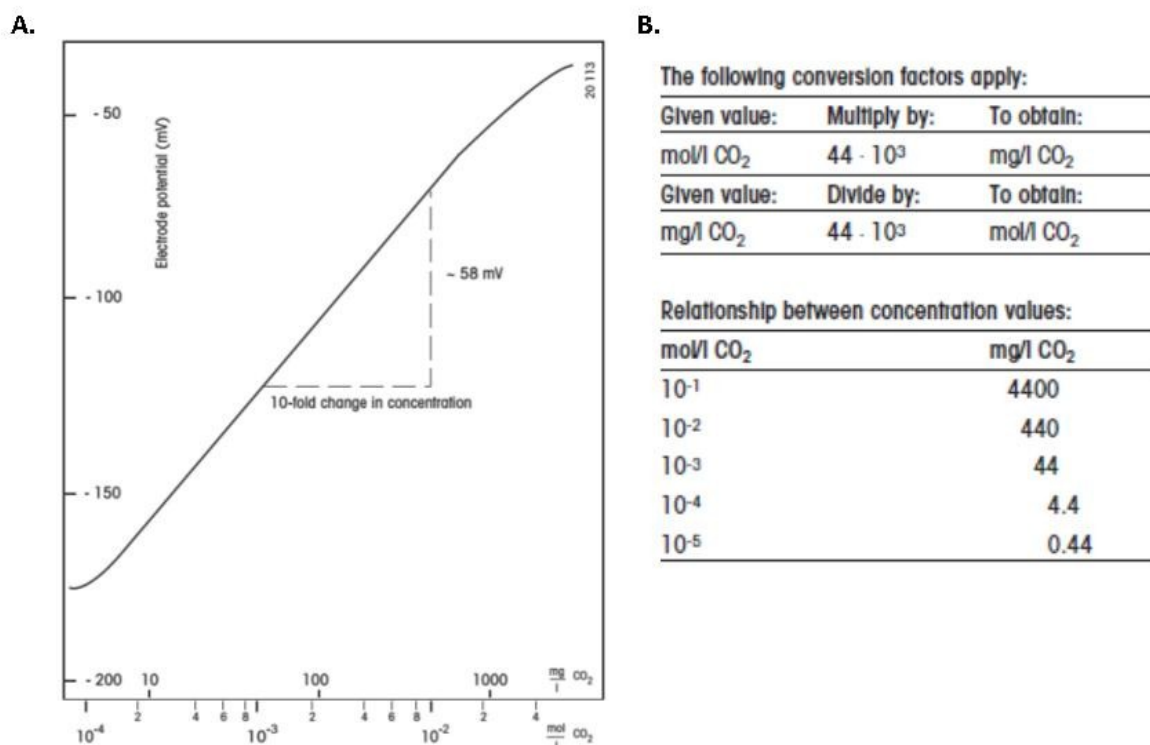


Fig. 26: A) Typical calibration curve for CO_2 electrode. B) Conversion tables. Modified from www.mt.com.

CHEMICAL SOLUTION

- Electrolyte solution as supplied with the CO_2 membrane kit (Ordering No. 51 340 015).
- Deionized water for preparing standard solutions.
- 32% HCl for pH adjustment.
- Standard 20 μM solution of NaHCO_3 and Na_2CO_3 (78.24 mg of NaHCO_3 and 98.59 mg of Na_2CO_3 in final volume of 200 ml of deionized water). The calibration solution can be used immediately or can be stored in closed air tight vials for longer time and future calibrations.
- N_2 gas source for CO_2 removal from solutions used for calibration.
- CO_2 electrode storage solution. Prepare as follows: Dissolve 5.8 g sodium chloride (NaCl) in about 200 ml deionized water and make up to 1000 ml.

CALIBRATION PROCEDURE

- Calibration and sample solutions should always be kept at the same temperature (if other vessel than PBR vessel is used for calibration it should be thermally insulated from possible heating by the magnetic stirrer). If the PBR vessel is used the solution within the vessel should be maintained at the temperature of the experiments.
- All measurements should be carried out under the same stirring conditions, i.e. identical stirring bars, identical distance between electrodes and stirring bars, etc.
- When not in use, the CO₂ electrode should always be kept immersed in the storage solution.
- In all calibration and sample solutions the ionic strength should be the same, and should not exceed a maximum value of 1.0 mol/l. During the calibration the pH value should be less than 4.0. This is achieved by adding HCl to the solution in the vessel during calibration resulting in release of all of the CO₂ available from the calibration solution.
- The electrode should not be immersed to a depth greater than the length of the membrane module, i.e. not more than 6 cm.
- During the calibration procedure the photobioreactor vessels should be completely filled with liquid and be closed to avoid any CO₂ gas loss from the system.
- If the calibration of the CO₂ probe is done in a different vessel than the photobioreactor vessel, please ensure that it is done at the temperature of the experiment, with good stirring and in a container that can be closed to the atmosphere to prevent loss of CO₂ gas.

DETERMINING CARBON DIOXIDE CONTENT

- Prepare deionized or distilled water. Adjust pH of distilled water to pH=3 with HCl and place it overnight in 90 °C water bath to remove any CO₂ gas remaining in the water (please note: Keep the lid of the container loose to allow the CO₂ to escape from the bottle while in water bath).
- After removal of deionized water from the water bath use the N₂ gas and bubble the deionized water for at least three hours while it is coming to experimental temperature.
- Assemble the electrode according to manufacturer's guidelines and place it in the vessel.
- Weigh out the required amounts of NaHCO₃ and Na₂CO₃ as mentioned above for 20 µM concentration and dry it for 2 hours in 200 °C in the drier before preparing the standard solution with deionized water. Keep the standard solutions closed.
- Determine the maximum volume of the vessel that will be used for the calibration. To do this, take the vessel which will be used for the calibration and fill it completely with water. Assemble the vessel with the lid and all the sensors that will be used in the experiment. Note that all the sensors submerged in the vessel will displace some of the volume of the liquid. Determine the exact volume by weighing the water filled vessel and subtracting the weight of the vessel with no water. Assuming density of 1 g/L for water, calculate the volume from the weight of the water. Alternatively, pour water out of the vessel into a cylinder to measure the volume. In general for 400 ml vessel when 2 sensors are used volume is around 475 ml, when 3 sensors are used the volume is around 465 ml. For 1000 ml vessel, maximum volume with two sensors is around 1200 ml.

5.Optional Accessories/Components

- Section Correction table for CO₂ calibration on page 39 includes a table which lists the volume of standard calibration solution that should be added to the vessel during calibration to obtain 20 µM solution of CO₂. The volume depends on the total volume of the vessel.
- Switch ON the PBR FMT150 and place the empty vessel in its holding place. Install the lid of the vessel with the sensor and other ports closed off. Ensure the stirring bar is placed inside the vessel and the magnetic stirrer is installed on the outside of the vessel. Fill the vessel with deionized water prepared overnight as described above. Use the N₂ gas to bubble the solution and remove any CO₂ that may have entered the water during preparations for the calibration (note that CO₂ is very soluble in water and extra precaution has to be taken to maintain the water CO₂ free). Allow the N₂ gas to escape through one of the ports like the waste port (keep it open during bubbling with N₂). In the meantime prepare additional 50 ml of water in falcon tube and bubble it through with N₂. This CO₂ free water is used for final removal of any residual air in the vessel prior the calibration itself.
- Connect the CO₂ electrode with PBR and start monitoring the raw data readings in the falcon tube (these will be visible in the idle mode). Signal around 30000 is in the range of background noise sensitivity of the CO₂ electrode. Once the calibration constants are determined these values will be changed to provide dCO₂ readings in correct units (µM or mg/L).
- Measure the CO₂ signal in the deionized water in the falcon tube. When the signal is stable in range around 30000 remove the sensor and close the tube. Insert the CO₂ sensor into the PBR vessel (calibration vessel) and check it for any gas bubbles that may be adhering to the membrane. Remove any bubbles by gently tapping the electrode body with the fingers. Check the signal, when values around 30000 are reached remove the N₂ gas supply tubing (supplying N₂ gas at present) and pour the CO₂ free water from falcon tube into the vessel. The vessel must be completely full. Unplug the waste tubing from the vessel and close all the inlets and outlets with LUER connectors.
- Define the calibration range required, preferably choosing concentrations which bracket the concentrations expected from the sample solution. When calibrating for the first time, at least three calibration points should be used. Table with volumes of stock calibration solution that should be added to the vessel to reach 20 µM dCO₂ concentration, based on maximum volume of the given vessel, are shown in Chapter . Please note that for example 1 ml of the calibration solution added to the vessel with volume of 465 ml results in final concentration 20 µM of dissolved CO₂ (dCO₂). If concentration of 100 µM dCO₂ is required 5 ml of calibration solution should be added to the vessel with volume of 465 ml.
- When the background CO₂ sensor signal is stable pipette the given volume of the calibration solution into the vessel through one of the port inlets. After addition of the calibration solution, the vessel should be tightly closed to avoid any loss of CO₂ from the vessel to the surrounding air. Make sure that the magnetic stirring is continuously operating during the time course of the calibration. 20 µM concentration of dissolved CO₂ corresponds approximately to 590 ppm of CO₂. Example of concentration conversion for the calibration solution is shown in Table 6.
- Read out the CO₂ signal in software and wait until the equilibrium in the signal is reached (it takes about 4 hours). Once the data reading has stabilized, record the final constant value and use it in preparing the calibration curve.
- When equilibrium is reached, add another amount of calibration solution to obtain next calibration step. For example if second calibration step with concentration of 50 µM dCO₂ is required, add another 1.5 ml of 20 µM calibration solution if vessel with total volume of 465 ml is used for total of 2.5 ml required to obtain 50 µM dCO₂.

5.Optional Accessories/Components

- **Important:** Please monitor the pH of the solution over the time course of the calibration to ensure that it remains at 4 or lower. Add more HCl into the solution if pH increases above 4. At pH above 4 dCO_2 will be converted back into bicarbonate and will no longer be measured by the probe (Fig. 27).

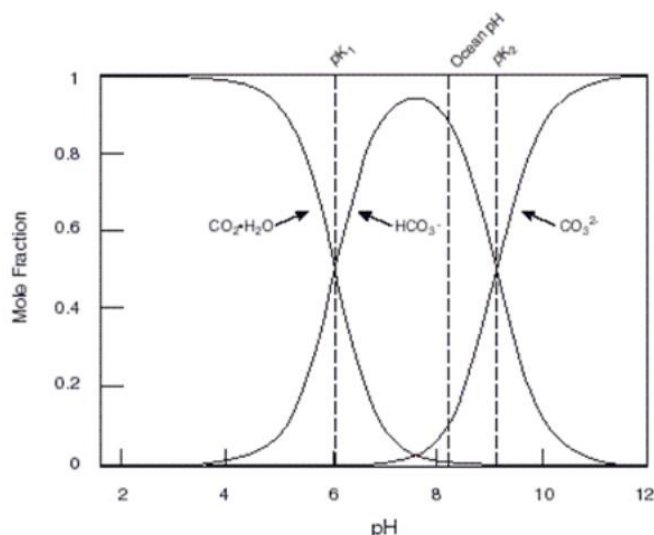


Fig. 27: Carbon Dioxide – Carbonic Acid Equilibrium.

- CO_2 calibration can be performed by PBR software. Please refer to the PBR Software Manual.
- The frequency of recalibration operations depends on the degree of accuracy required and on the measuring conditions. The CO_2 probe should be re-calibrated when the experimental temperature is different from the one used during the calibration. If temperature is not changed, we recommend re-calibration every three months or at the least one point calibration check for verification purposes.

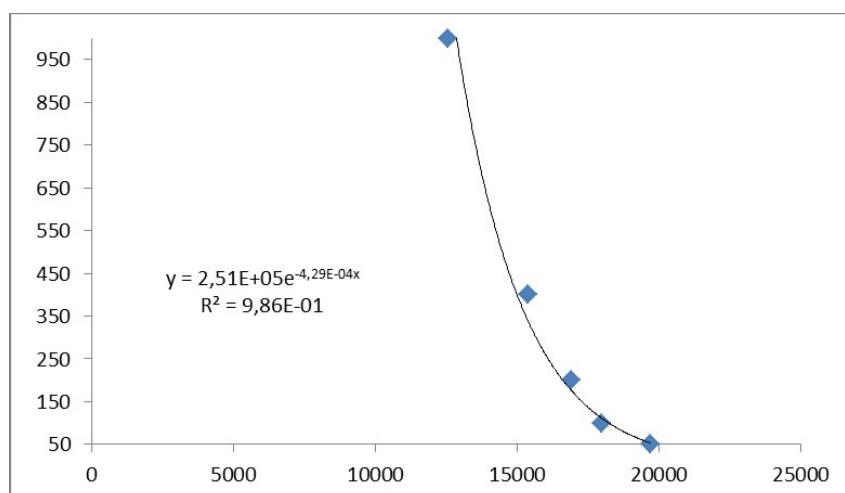


Fig. 28: Typical CO_2 calibration curve. Right: Exponential fit equation with depicted constants a and b.

$$y = \overset{\text{b}}{2,51\text{E}+05} \text{e}^{\overset{\text{a}}{-4,29\text{E}-04x}}$$

$$R^2 = 9,86\text{E}-01$$

$$y = b \cdot \text{Exp}(a)$$

$$b = 250608$$

$$a = -0.00042923$$

Example of resulting μM concentration of dissolved CO_2 in the vessel when air with 400 to 15000 ppm CO_2 content is used to bubble the water. Please note that the values are only for demonstration purposes and will depend on temperature, atmospheric pressure and gas flow rate.

5.Optional Accessories/Components

Bubbled gas	Temp	dCO ₂ (μM)	dCO ₂ (mg/L)
400 ppm CO ₂	25 °C	13.6	0.6
5000 ppm CO ₂	25 °C	170	7.48
10000 ppm CO ₂	25 °C	340	14.96
15000 ppm CO ₂	25 °C	510	22.44

Table 6: CO₂ calibration example

CORRECTION TABLE FOR CO₂ CALIBRATION

Correction table for the volume of calibration solution to be added into the vessel for the calibration step in respect of maximum vessel volume.

Note: 1 ml of calibration solution added to the 400 ml cuvette with maximum volume of 465 ml results in final concentration of 20μM dCO₂ in the vessel.

FNT150 400 ml		FNT150 1000 ml	
ml DIC free H ₂ O in cuvette	ml calibration solution	ml DIC free H ₂ O in cuvette	ml calibration solution
450	0.968	1185	2.548
451	0.970	1186	2.551
452	0.972	1187	2.553
453	0.974	1188	2.555
454	0.976	1189	2.557
455	0.978	1190	2.559
456	0.981	1191	2.561
457	0.983	1192	2.563
458	0.985	1193	2.566
459	0.987	1194	2.568
460	0.989	1195	2.570
461	0.991	1196	2.572
462	0.994	1197	2.574
463	0.996	1198	2.576
464	0.998	1199	2.578
465	1.000	1200	2.581
466	1.002	1201	2.583
467	1.004	1202	2.585
468	1.006	1203	2.587
469	1.009	1204	2.589
470	1.011	1205	2.591
471	1.013	1206	2.594
472	1.015	1207	2.596
473	1.017	1208	2.598
474	1.019	1209	2.600
475	1.022	1210	2.602
476	1.024	1211	2.604
477	1.026	1212	2.606
478	1.028	1213	2.609
479	1.030	1214	2.611
480	1.032	1215	2.613
481	1.034	1216	2.615
482	1.037	1217	2.617
483	1.039	1218	2.619
484	1.041	1219	2.622

Fig. 29: Correction table for the volume of calibration

5.6 MAGNETIC STIRRER

The magnetic stirrer (Fig. 30) rotates a teflon-coated magnetic bar in a vertical plane of the front glass panel inside the Photobioreactor. The speed of rotation is adjustable manually by the front panel of the control unit or by the PBR software.

The magnetic stirrer module is designed for homogenous mixing of the culture inside the cultivation vessel. The stirrer is essential when the agitation by air or gas bubbles cannot be used, e.g. when the photosynthetic oxygen evolution or respiratory oxygen uptake are measured or when growing the culture anaerobically without gas supply. It can be also used to reduce cell sedimentation and clumping in addition to agitation by gas bubbling. The magnetic stirrer is highly recommended when using the O₂ probe and CO₂ probe – very good stirring is essential for accurate measurements of these probes. **Note:** The magnetic stirrer module cannot be used simultaneously with an external light. One or the other can be installed at one time as they both fit on the outside of the vessel.

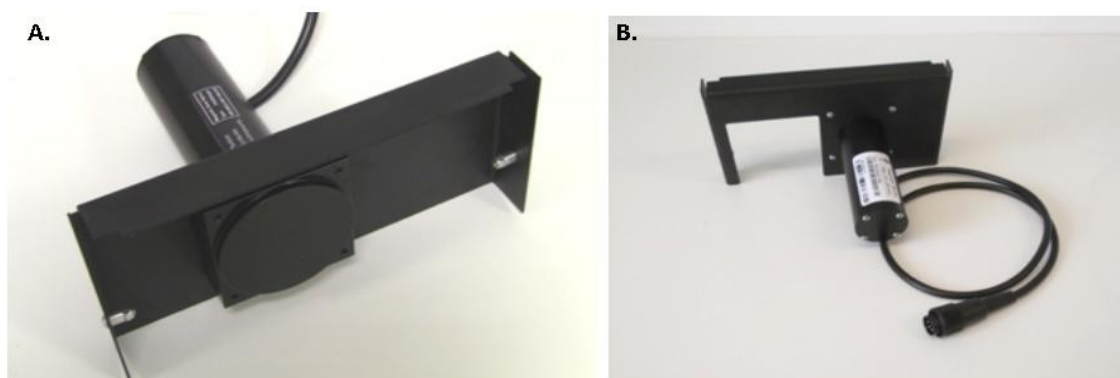


Fig. 30: Magnetic stirrer. A) FMT 150.1 version; B) FMT 150.2 version.

Note: Magnetic stirrer cannot be used simultaneously with an external light panel as they both fit on the outside of the vessel.

5.6.1 INSTALLATION

Follow the instructions below to install the magnetic stirrer:

- Magnetic stirrer has two screws positioned on each side of the inner holder as shown with blue arrows in Fig. 31A.
- Position the stirrer with the inner side towards the PBR front window by positioning the screws on the inner side of the stirrer as shown in Fig. 31A.
- Connect the stirrer cable to AUX1 connector on the back of the Photobioreactor (Fig. 31C).

Important note: Please note that the communication connection scheme with the PBR FMT150 varies depending on the complete set-up of the system. Please refer to page 20 Fig. 9 for schematic representation of different connection set ups based on the system composition.

- Insert the stirring bar inside the Photobioreactor cultivation vessel during cuvette assembly as described on page 24.
- The function of the stirrer can be controlled manually by the front panel of the control unit or by the PBR software.

5.Optional Accessories/Components

- For manual operation via front panel please refer to chapter 8.1 on page 59 for more details. Briefly go to menu Setting→PWM_Mode. Here the stirrer can be turned ON/OFF and its speed can be adjusted in percentage of total power (10 % low power, 100 % high power) as shown in Fig. 31D.

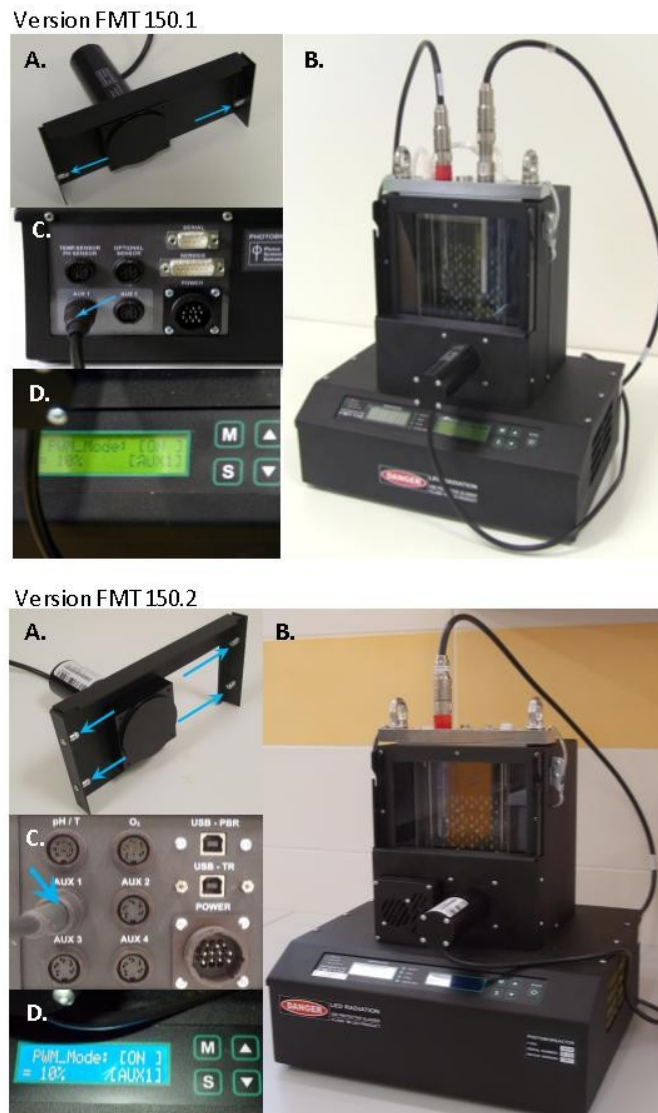


Fig. 31: Installation of the magnetic stirrer. A) Screws for fixing the stirrer to the front side of the PBR main body. B) Magnetic stirrer is fastened in the correct position to the front part of the PBR. C) Attaching of the communication cable to the “AUX 1” connector at the rear side of the PBR. D) Manual Stirrer control via the PWM_Mode.

5.7 PWM PUMP

PWM pump (Fig. 32) can be used for pumping liquid or gas into the cultivation vessel. It can be regulated within a range of 10-100 % of total output. The pump has diverse uses:

- Controlling air bubbling in the cultivation vessel
- Single gas input into the vessel

5.Optional Accessories/Components

3. When connected to the vessel inlet and outlet (closed flow mode), it can be used for suspension mixing (bubbling) in anaerobic conditions

Please note that PWM pump cannot be used simultaneously with magnetic stirrer as both need to be connected to AUX1 on the rear side of the photobioreactor.



Fig. 32: PWM Pump

5.7.1 INSTALLATION

Instructions below explain connection of the PWM Pump with the Photobioreactor FMT150:

- The function of the PWM pump can be controlled manually by the front panel of the control unit and remotely via Remote Control software for PBR FMT150. For manual operation via front panel please refer to appendix on page 59 for more explanation. Briefly go to menu **Setting** → **PWM_Mode**. Here the PWM pump can be turned ON/OFF and its performance can be adjusted in percentage of total power (10 % low power, 90 % high power).
- PWM Pump when connected to the PBR has to be set for REMOTE MODE operation to be directly controlled and operated via Remote Control software for PBR FMT150. This pump can also be used in a manual mode as a stand-alone instrument. **Note:** When connected to the PBR and used In REMOTE MODE PWM Pump can replace the function of the Bubble Interruption Valve.
- For REMOTE mode plug the grey connector cable to the PWM pump rear and connect it with the AUX2 connector of the PBR FMT150. Set the speed control of the PWM Pump to **REMOTE MODE** position (Fig. 33B). PWM Pump is now controlled via PBR FMT150 software.
- For the stand-alone MANUAL mode operation connect the PWM Pump power supply with the PWM Pump and plug to AC outlet. The flow rate can be controlled manually with the speed control knob on the front panel.

Important: If MANUAL mode of the PWM Pump is used for controlling air bubbling in the cultivation vessel, bubble interruption valve must be used to interrupt the bubbling during the measurements.

- Connect the short silicone tube to the **IN** port on the rear panel of the PWM Pump. (Note: This improves supply of homogenous gas flow to the pump). Connect the silicone tubing between the **OUT** port on the PWM Pump rear panel with the humidifier bottle (Fig. 33C) or with the bubble interruption valve (Fig. 18) depending on the configuration used.

5.Optional Accessories/Components

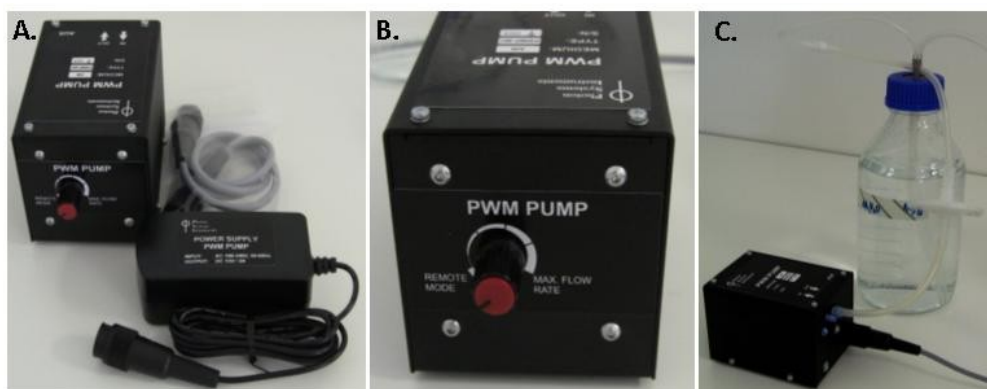


Fig. 33: PWM Pump. A) PWM Pump components. B) Front panel with the speed control knob in REMOTE MODE position. C) Connection of the PWM Pump with the humidifier bottle.

5.8 GAS MIXING SYSTEM GMS 150

Gas Mixing System GMS 150 (Fig. 34) can produce precise mixtures of up to 4 different gasses. The flows of the individual input gases are measured by thermal mass flow meters and adjusted by integrated mass flow controllers. The gas mixture is thoroughly homogenized, before leaving the device. The input and output gas connectors are of Prestolok type allowing fast and secure connection to a variety of tubes.

The gas mixer can have up to four channels (gasses). The standard version of the system includes two channels:

- Channel 1 supplies Air-N₂ mixture or Air and has flow rate 40-2000 ml/min
- Channel 2 supplies CO₂ and has flow rate in range 0.8-40 ml/min

Please note that when one GMS is used to supply gas to more than one photobioreactor, the use of one PWM pump per photobioreactor is required to supply portion of the gas from the gas mixing system to the bioreactor without affecting the gas flow among the bioreactors.



Fig. 34: GMS 150.

The GMS 150 mass flow meters and controllers can operate gas flows ranging from ml/min to tens of litres/min. Typically, the controllers of GMS 150 operate in the lowest range 0-8 ml/min and in the highest range 0-2500 ml/min.

The user can define the required gas mixture either by setting the flows of the individual gasses (e.g., 980 ml/min of N₂ and 20 ml/min of CO₂) or by setting the required relative composition of the gas mixture (2% CO₂) and the mixture gas flow (1000 ml/min). The GMS 150 can be used as a stand-alone instrument with other applications or as an additional and optional component of the Photobioreactor FMT150.

The GMS 150 can be controlled also by PBR software. Detail description of the High-precision Gas Mixing system installation and operation can be found in the manual provided together with the GAS Mixing (GM) System and in the PBR Software Manual.

5.8.1 INSTALLATION

Follow the instructions below to connect the Gas Mixing System with the PBR FMT150:

- Connect the GM System with the gas sources (n°1 in Fig. 35A) and set the desired composition of the gas via the front panel display of the GMS 150.
- Connect the output GM system tubing (n°2 in Fig. 35A) with the bubble interruption valve (n°3 in Fig. 35A). Bubble interruption valve is further connected as shown and described in Fig. 18.
- When the GM System is connected to the PBR FMT150 the vessel lid assembly scheme (Fig. 35B) is same as for standard air installation as described on page 24.
- When more than one PBR FMT150 units are connected to the same GM system the set up of such connection scheme is shown in Fig. 35D. For connecting more PBR FMT150 units to one GMS 150 system the customer needs to purchase one PWM pump for each PBR FMT150 unit.

Output flow tubing from the GMS 150 should be connected with the individual PWM pumps such that the final sum of the GMS 150 flow rate (schematically described in Fig. 35D as V_{GMS}) is bigger than the sum of each PWM pump flow rate. **Note:** that the end of the output flow tubing from the GMS 150 to which the individual PWM pumps are connected must be open to allow release of residual air from the tubing.

For installation of PWM pumps and their connection to PBR FMT150 please refer to chapter 5.7 on page 41.

5.Optional Accessories/Components

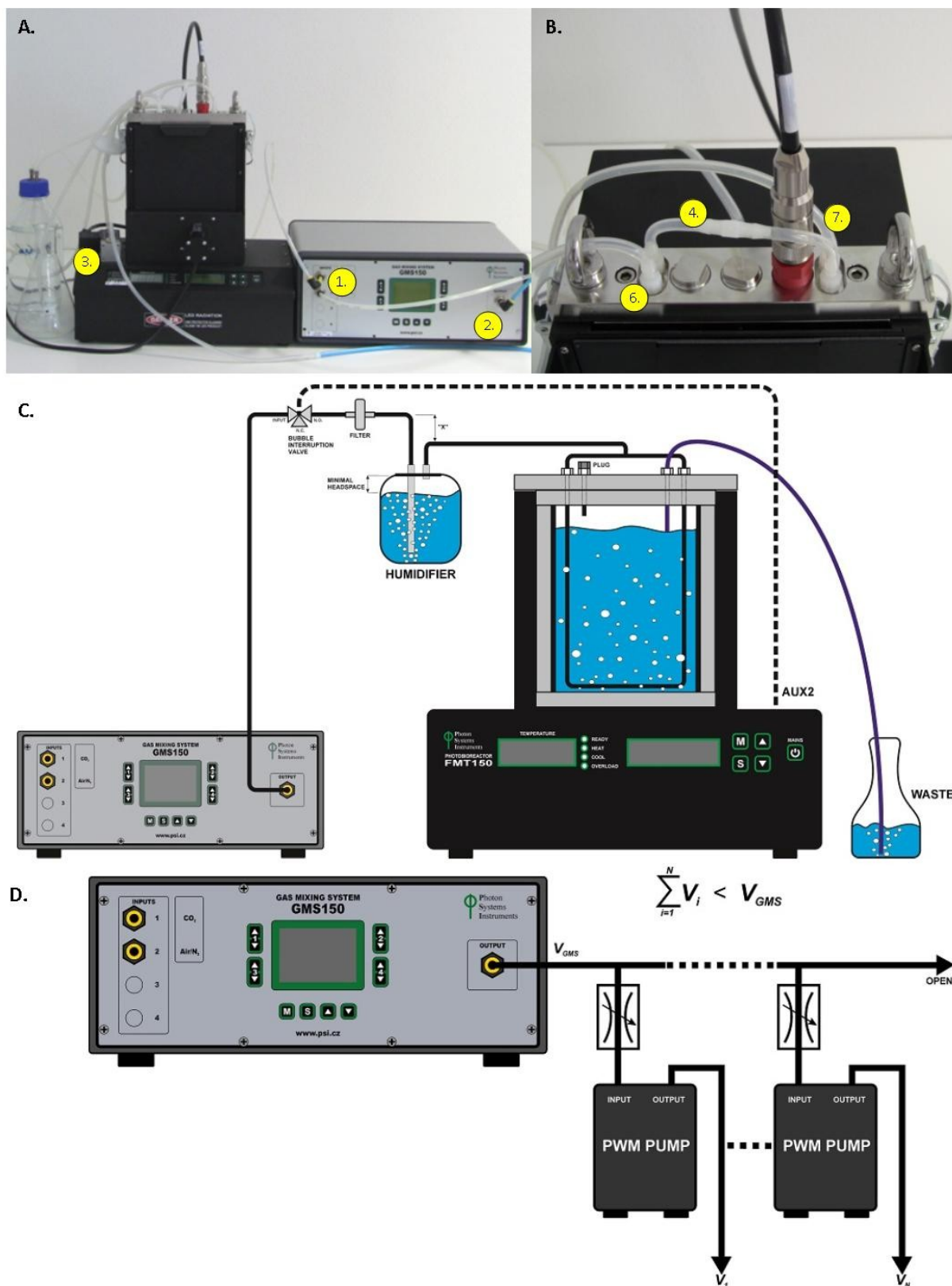


Fig. 35: GM system connection with the PBR FMT150. A) Mixed gas of given composition (1) is pumped from GM System via (2) to the PBR FMT150 via air interruption valve (3). B) Vessel lid assembly. (4) Air distribution tube, (7) Medium outlet, (6) Sampling tube. C) Scheme for the connection of GM with PBR FMT150. D) Scheme for connecting more PBR FMT150 units to one GM System. V_i refers to air flow to one PBR unit. V_{GMS} refers to total air flow output of GM unit.

5.9 GAS ANALYSIS SYSTEM GAS 150

The GAS 150 (Fig. 36) enables measurements of CO₂ with high precision at the cultivation vessel gas output (from the head space above the culture). The GAS 150 can be used as a stand-alone gas analysis system or it can be incorporated into a system with Photobioreactor FMT150.



Fig. 36: GAS 150.

5.9.1 INSTALLATION

Follow the instructions below to connect the Gas Analysis System 150 (GAS 150) with the PBR FMT150:

- Connect the GAS 150 with the AC power cord which is part of the accessory GAS 150 package (Fig. 37A) and plug to electrical outlet.
- Assemble the dehumidifier as shown schematically in Fig. 37B-F and place it into the holder as shown in Fig. 37E-F. **Note:** Dehumidifier is used to prevent water vapour from the cultivation vessel from entering the GAS 150 system.
- Connect the GAS 150 to the gas source (n°2 in Fig. 38). Here the gas source comes from the Gas Mixing System (GMS 150)(n° 1).
- Connect the **OUTPUT** GMS tubing (n°1 in Fig. 38) with **GAS** input (n°2 in Fig. 38) via a T-parker connector with a check valve (n°8 in Table 3) (Fig. 38 blue arrow).
- Using parker tubing and silicone tubing with Luer Lock Fittings connect Gas outlet of the GAS (n°3 in Fig. 38) via bubble humidifier bottle (n°4 in Fig. 38) to PBR FMT150.

5.Optional Accessories/Components

- Outlet from the bubble humidifier bottle is connected to the vessel lid as described previously on page 23.

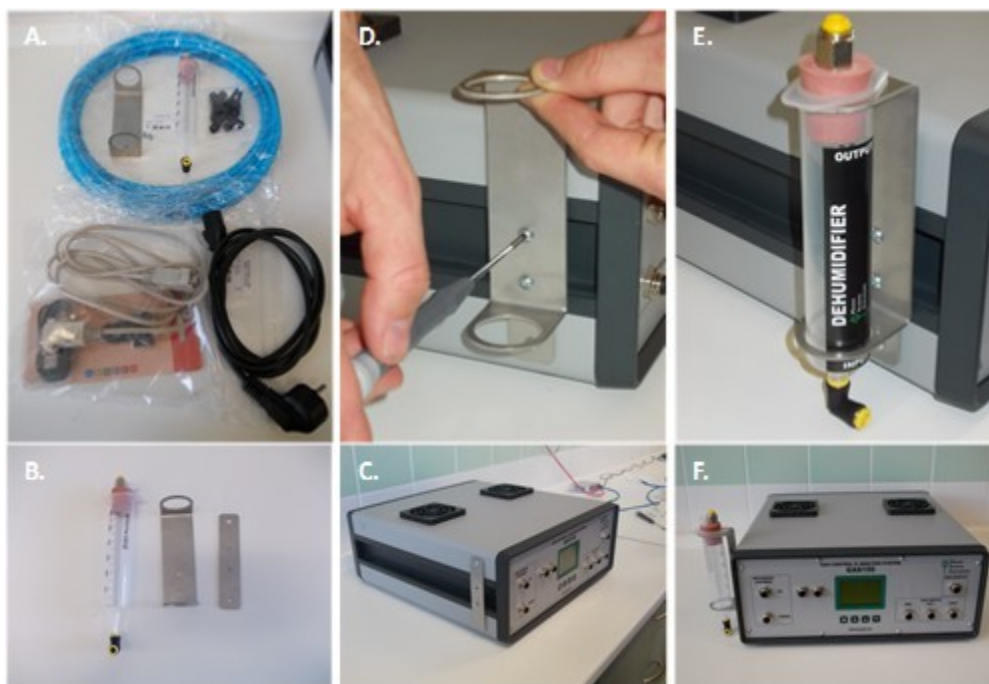


Fig. 37: GAS 150 dehumidifier assembly.

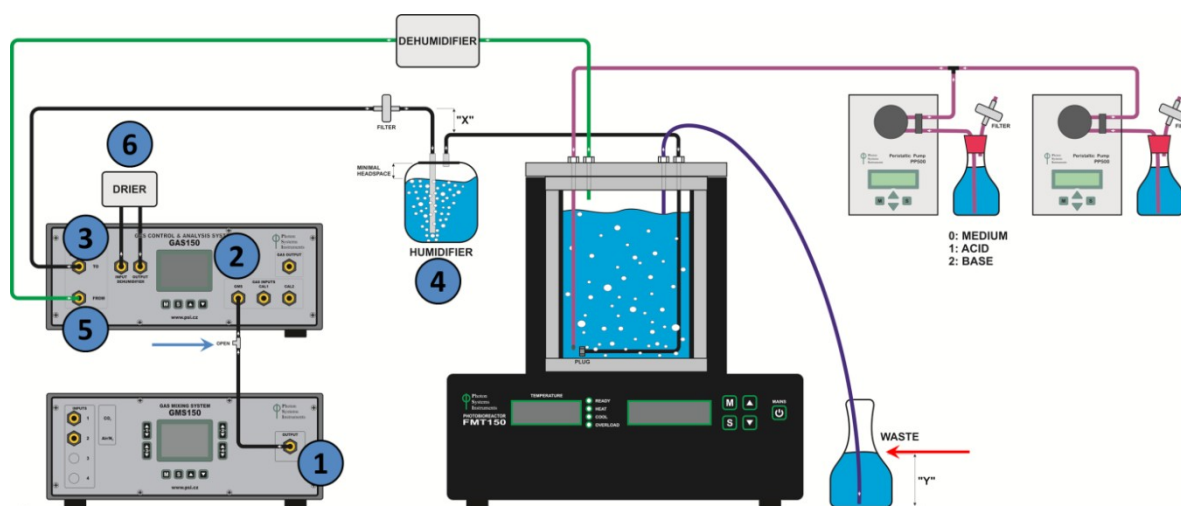


Fig. 38: Schematic representation of connection scheme when GAS 150 system is connected to PBR FMT150 together with GMS 150 and peristaltic pumps. Numbers correspond to: 1-GMS 150 output; 2-GAS 150 input for mixed air from GMS 150; 3-GAS output for bringing the mixed air to humidifier bottle (4) and to the PBR FMT150. 5-Input for gas from the cultivation vessel to GAS 150. 6-Air from the vessel passes through dehumidifier prior CO₂ concentration analysis.

Important note: If peristaltic pump in chemostat or turbidostat mode is connected with the PBR FMT150 together with GAS System the vessel lid configuration with the tubing assembly is different as compared to setup with no chemostat/turbidostat module and no GAS. The set up is shown in Fig. 39B when standard aeration U-tube is provided.

Gas dispenser tubing (A. in Fig. 39A) is disconnected at the silicone joint bottom part and closed with Luer Lock Ring Plug (n°4 in Table 3; red arrow in Fig. 39A). As a result one long metal tube is disconnected from the air dispenser

5.Optional Accessories/Components

tubing (D. in Fig. 39A) and is used for peristaltic pump connection. The rest of the vessel lid and the cultivation vessel is assembled as in standard version. See Fig. 39B for detail view of the vessel lid assembly.

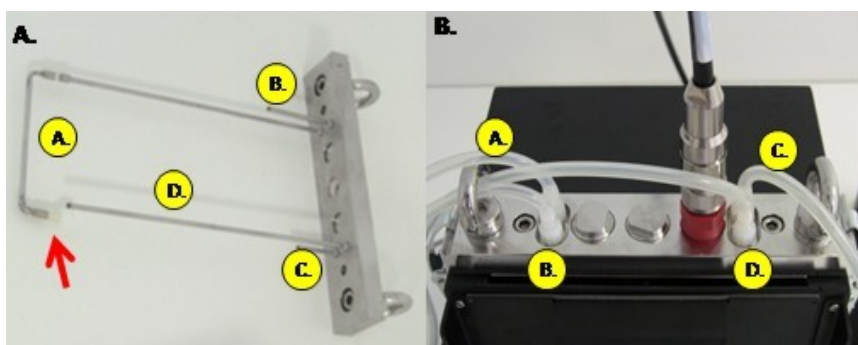


Fig. 39: Vessel lid assembly when GAS 150 system module is connected to PBR FMT150. A) Lid assembly when peristaltic pumps are connected to PBR FMT150 in parallel with GAS 150 and standard aeration U-tube is used. B) Top view of vessel lid when peristaltic pumps are connected to PBR FMT150 in parallel with GAS 150. Letters in yellow circles refer to: A-Aeration tubing; B-medium outlet; C-gas sampling tube; D-tube for peristaltic pump connection.

Two different versions of aeration U-tube can be provided, vessel lid with standard aeration U-tubing is shown in Fig. 40A and vessel lid with GAS aeration U-tubing is shown in Fig. 40C. In Fig. 40B standard aeration U-tubing modified for GAS connection together with peristaltic pump as described above is shown.

If GAS 150 module is purchased both types of aeration U-tube are provided. To change the two types of aeration tubing please follow the schematic representation of individual steps as shown in Fig. 40.

- Connect the gas outlet of the cultivation vessel via the silicone tubing (C in Fig. 39A,B) via parker tubing with the gas inlet of the GAS (labelled **FROM**) (n°5 in Fig. 38).

Important note: the waste bottle for medium waste outlet (B in Fig. 39B) should be filled up to 1/3 of its volume (red arrow in Fig. 38) with water to ensure that the air from the vessel goes always via GAS 150 and thus is not escaping through the waste tubing out of the closed system due to the pressure imbalance.

- Switch ON the GAS System and follow the Instruction Manual for further operation of the System.
- GAS system can be operated from the GAS front panel display as described in the GAS Instruction Manual. GAS System also can be monitored by PBR software.

Note: Please note that when using the GAS system with the PBR the bubble interruption valve is not necessary as the GAS can perform interruption of bubbling during measurements of parameters.

5.Optional Accessories/Components

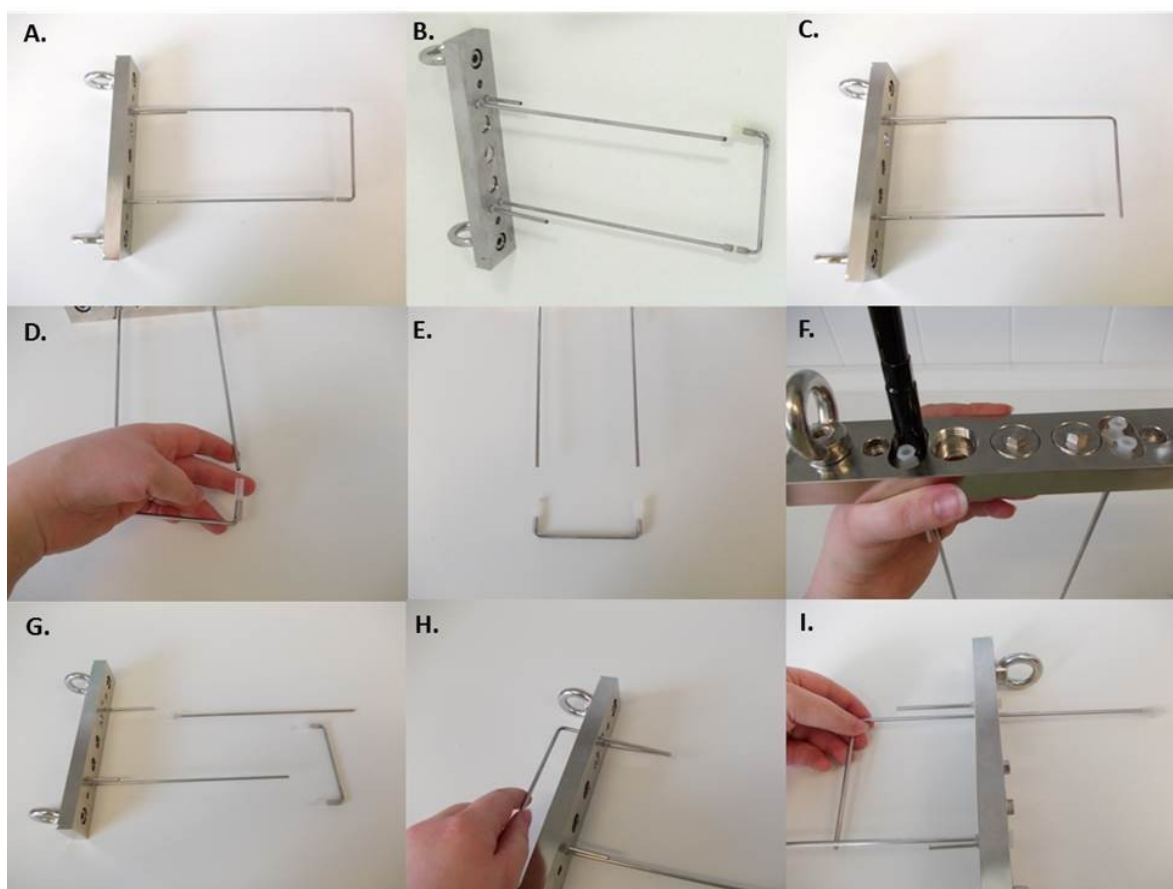


Fig. 40: Vessel lid with standard and GAS aeration U-tubing. Pictures D-I show individual steps for changing the standard aeration tubing and exchange with GAS aeration U-tubing. Note: Use spanner (n°14 in table 1) to release the valve plastic connectors from the vessel lid.

5.10 PERISTALTIC PUMP PP500

Peristaltic pump PP500 (Fig. 41) can be used in chemostat, turbidostat or standalone cultivation mode. Please note that PBR software is required to use the pump in turbidostatic or chemostatic mode.

Note: the operation of the peristaltic pump cannot be controlled by the front panel of the PBR FMT150 control unit. Without the PBR software, the operation of peristaltic pump is controlled solely by the Peristaltic pump Control display.

Important note: It is essential to define if the pump is serving for turbidostatic or chemostatic module or if it is working in standalone mode. It is done in the Peristaltic Pump control display – go to **Menu>Setting>Pump-ID [x]** and set the required mode as described below:

- [0] should be set for turbidostat mode
- [1] should be set for chemostat mode – Acid
- [2] should be set for chemostat mode – Base
- [3-7] should be set for standalone mode

Important note: the pumps connected to the FMT150 must have unique IDs, otherwise the behavior of pumps is unpredictable.

5.Optional Accessories/Components

Turbidostat mode – the Photobioreactor optical sensing unit monitors the optical density (OD680, OD720/OD735) of the culture. The Peristaltic Pump adds fresh medium to maintain configured OD levels according to protocol devined in PBR software.

Chemostat mode – the pH value of the cultivation media can be continuously adjusted as either acid or base is added to the cultivation vessel to keep the pH level at given value according to protocol defined in PBR software.

Standalone mode – controlled manually via the peristaltic pump control display or by PBR software. For manual control please refer to the Peristaltic Pump operation manual.

The pump allows variable flow-rate from 10 to 5600 µl/min and this flow rate is set manually on the pump control display or via PBR software for each pump.



Fig. 41: Peristaltic Pump PP500.

The Peristaltic Pump consists of two main parts – the Peristaltic Pump PP500 and the small stabilized power supply. Detail description of the pump can be found in the manual provided together with the peristaltic pump.

5.10.1 INSTALLATION

Follow the instructions below to install and operate the peristaltic pump:

- First install the tubing connection with the PBR FMT150.
- Attach provided short tube with Male Luer Lock Fittings (n°1 in Table 3) to the Peristaltic Pump PP500. Insert one end of the tube in the holder and wind the tube as shown in the figure below (Fig. 42A,B). Insert the other end of the tube into the holder (Fig. 42B). Ensure that the end of the tubing is pushed all the way to the end of the luer lock fitting. If this is not done, small air leak may occur resulting in the pump not working properly.

5.Optional Accessories/Components

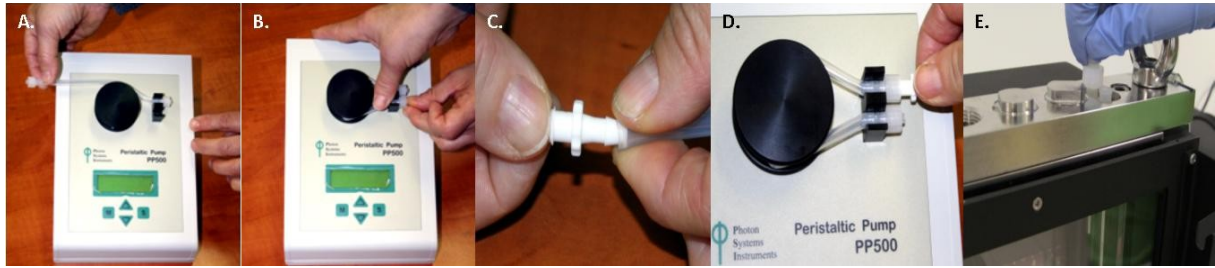


Fig. 42: Assembly of the peristaltic pump. A, B) Short tube is attached around the rotor wheel to the peristaltic pump. C, D) Adapter is attached to the silicone tubing and connected with the tubing of the peristaltic pump. E) Tubing from the peristaltic pump is connected with the cultivation vessel.

- Attach provided female luer connector to the silicone tube and connect it to the peristaltic pump as shown in Fig. 42C,D. Again ensuring that the tubing is pushed all the way to the end of the luer lock connector.
- Connect the free end of the silicone tube via Male Luer Lock Fitting with the lid of the cultivation vessel (Fig. 42E). The position for connection on the vessel lid corresponds to the position of outlet n°6 in Fig. 12C.
- Attach a new piece of tubing to another female luer connector and attach it to the Peristaltic Pump as shown in Fig. 43A).
- Place the other end of this tubing in the suspension or medium (Fig. 43B) depending on the application of the pump.

5.Optional Accessories/Components

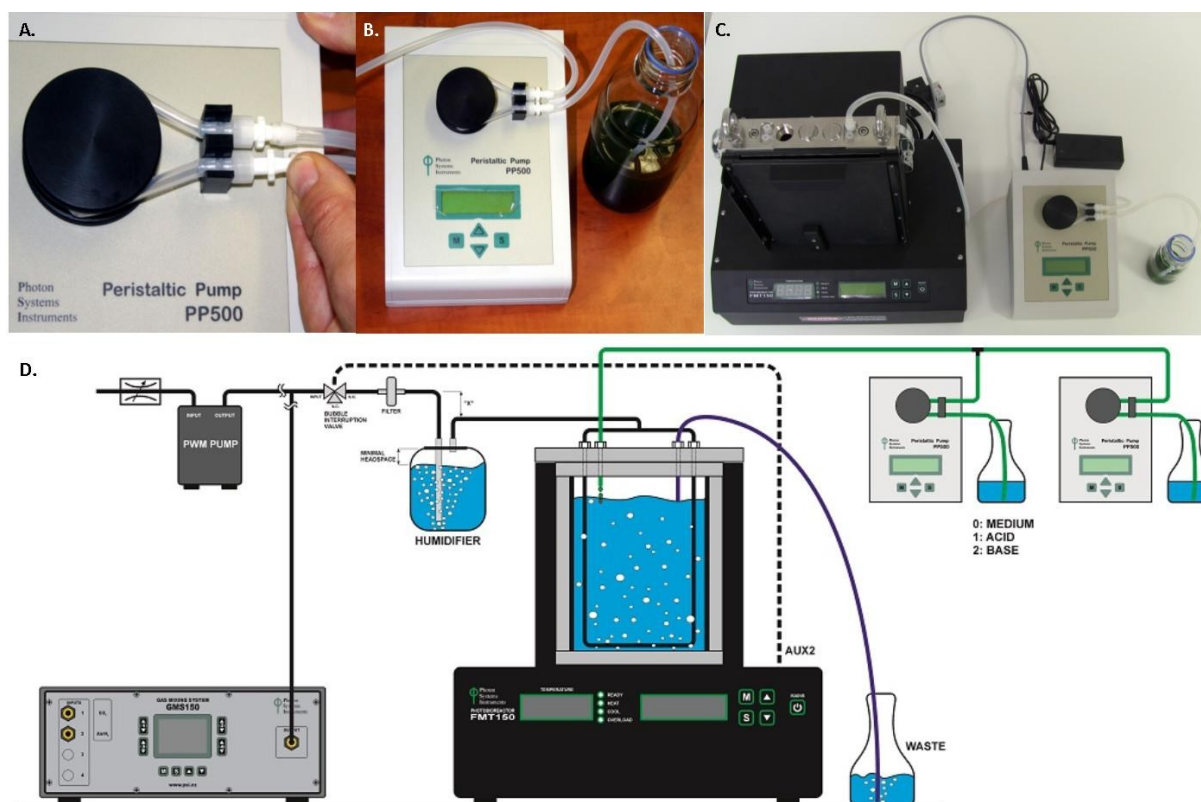


Fig. 43: Final assembly of the peristaltic pump. A) Second luer connector is attached. B) Connection of the silicone tubing with the culture for the cultivation. C) Fully installed peristaltic pump tubing. D) Schematic representation of PBR FMT150 unit connected to peristaltic pumps in chemostat and turbidostat mode and to GMS 150.

- Connect the silicone tubing from the peristaltic pump to the vessel lid. Peristaltic pump tubing is attached to the outlet for the sampling tube (n°6 in Fig. 12A,C). Please refer to Fig. 43D for schematic representation of the PBR FMT150 set-up when peristaltic pump module and GM module is included.

Important note: Please note that the vessel lid assembly is different if peristaltic pump is used together with the Gas Analysis module. Please refer to page 48 and Fig. 39 for further instructions.

- Install the wiring connections for the peristaltic pump as shown in Fig. 44.
- Connect the Photobioreactor and the Peristaltic Pump using provided communication cable.

Important note: Please note that the communication connection scheme with the PBR FMT150 varies depending on the complete set-up of the system. Please refer to page 20 Fig. 9 for schematic representation of different connection set ups based on the system composition.

- Plug one end of the communication cable into the “AUX IN” connector of the Peristaltic Pump (Fig. 44A).
- Plug the other end of the cable into the “AUX 1” or other available AUX connectors at the rear of the Photobioreactor (Fig. 44B). Please refer to page 20 for further information.
- Connect the Peristaltic Pump via the power supply cord into the “POWER +24V DC” connector of the Peristaltic Pump (Fig. 44C).
- See Fig. 44D for complete setup of the Peristaltic Pump.

5.Optional Accessories/Components

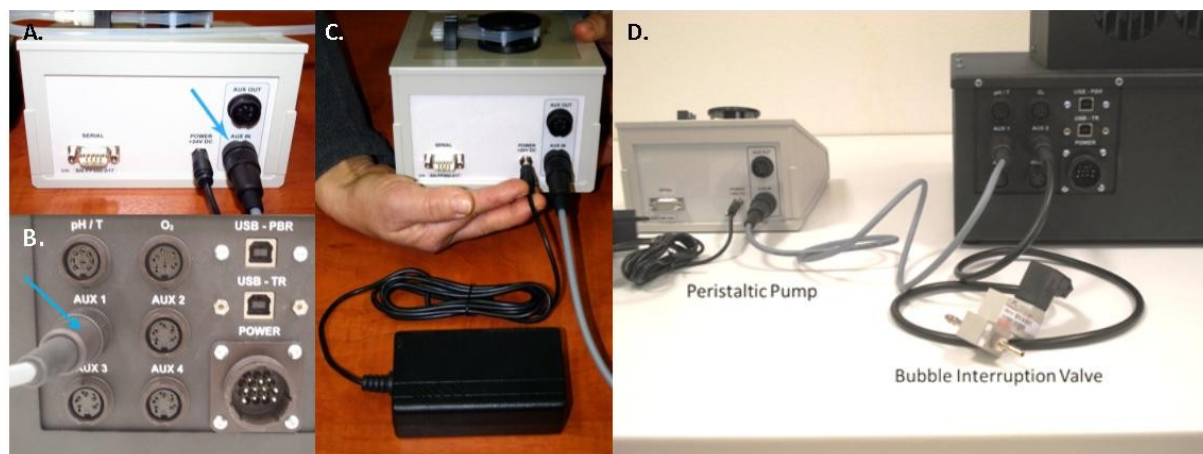


Fig. 44: Connection of peristaltic pump with PBR FMT150.2.

5.11 EXTERNAL LIGHT PANEL

The Photobioreactor can be supplemented with an additional external light panel that helps to accommodate specific needs of different organisms or to increase illumination intensity. External light panel is offered in three versions:

- single colour
- single colour with additional IR LEDs
- bicolour

See chapter 3.1.2 on page 8 for detail list of available versions. Please note that the external LED panel cannot be used simultaneously with the magnetic stirrer. One or the other can be used at one time. Following instructions are designated for PBR FMT150.1 version.

5.11.1 INSTALLATION

Follow instructions below to install and operate the external light panel:

- See the proper position of the holders in Fig. 45A.
- Position the Light Source by inserting its pivot joints into the respective slots in the holders – see Fig. 45B.
- Fig. 45C shows the proper position of the Light Source.

5.Optional Accessories/Components

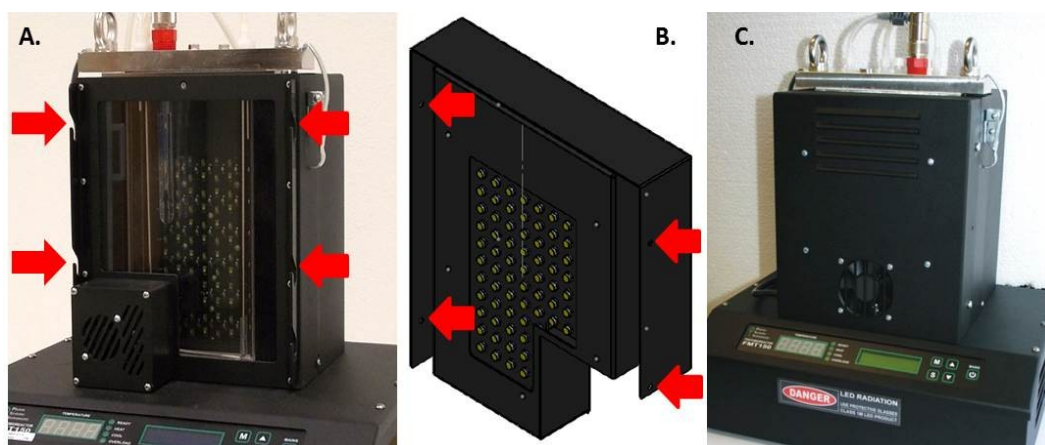


Fig. 45: Correct positioning of the external light panel and the final set up of the PBR with the external light source.

The function of the external light is controlled manually by the front panel of the PBR control unit or by PBR software. For manual operation via front panel please refer to chapter 8.1 on page 59.

5.12 ADDITIONAL SOFTWARE LICENSE

A separate license is required for each additional PBR software installation.

5.13 LIGHT UPGRADE

Increases intensity of actinic light in the Photobioreactor. The maximum reachable depends on light colour as listed on pg. 8.

5.14 SUPPLEMENTARY/REPLACEMENT COMPONENTS

5.14.1 SUPPLEMENTARY PH/T PROBE

Additional sensor probe to pH/Temperature module.

5.14.2 ADDITIONAL CABLE TO PROBES

Supplementary cable and connector for O₂ or pH/Temperature measuring probe.

5.14.3 SUPPLEMENTARY O₂ PROBE

Additional sensor probe to O₂ module.

5.14.4 O₂ MEMBRANE KIT

O₂ membrane replacement kit for O₂ probe. Includes: 4 membrane bodies, 1 O-ring set, 25 ml electrolyte.

5.14.5 CO₂ MEMBRANE KIT

CO₂ membrane replacement kit for CO₂ probe. Includes: 4 membrane bodies, 1 O-ring set, 25 ml electrolyte.

5.Optional Accessories/Components

5.14.6 SUPPLEMENTARY VESSEL (STANDARD MODEL)

Additional vessel, suitable for cultivation in the range of 18 – 50 °C. Made from stainless steel and glass, it includes the frame, lid and glass body. Manufactured in a 400 ml, 1000 ml or 3000 ml version.

5.14.7 ENHANCED VESSEL

Suitable for cultivation in the range of 0 – 70 °C. Made from nickel-plated brass covered with ceramic layer. Manufactured in a 400 ml, 1000 ml or 3000 ml version.

5.14.8 REPLACEMENT GLASS WINDOW FOR CULTIVATION VESSEL

Includes only the glass body (not the frame, base, or lid). Made of boiling glassware, thickness 3.3 mm. Delivered for 400 ml, 1000 ml or 3000 ml vessels.

5.14.9 GASKET KIT FOR CULTIVATION VESSEL

It includes 5 pcs Silicone gasket for vessel base, 5 pcs Silicone gasket for vessel lid, 15 pcs Silicone O-ring 75/3, 15 pcs Silicone O-ring 16/2. Delivered for 400 ml, 1000 ml or 3000 ml vessels.

6. CULTIVATION GUIDE

This section describes how to set up a standard Photobioreactor FMT150 and how to initiate cultivation of an algal culture with a PBR software.

6.1 SET UP THE COMPONENTS

1. First prepare the glass cultivation vessel and its lid. Follow the steps described in chapter 4.2.3 on page 24 to assemble the vessel. Cover the lid of the vessel with aluminium foil and prepare these components for autoclaving.

Important: Never close all three sensor holes with the screws. It is recommended to leave one sensor hole empty and cover the lid completely with aluminium. Provided sensors can be autoclaved but their lifetime is reduced.

2. Prepare the required medium for autoclaving (volume depends on the vessel capacity).
3. Prepare all silicone tubes and connectors necessary for gas inlet and gas/medium outlet for autoclaving.
4. Assemble bubble humidifier bottle as described on Fig. 17 on page 29 and prepare it for autoclaving.
5. Prepare glass or plastic funnel to be used for pouring of the autoclaved culture medium into the vessel for autoclaving.

Optional: Other components need to be autoclaved prior to experiment initiation if other optional components are used together with the standard PBR FMT150 set up. For example sampling tube and sampling syringe; additional medium for turbidostat mode; silicone tubes for peristaltic pump and/or magnetic stirring bar if magnetic stirrer is used.

6. Autoclave all components at 120 °C for 20 minutes or according to user's defined protocol.

6.2 SET UP THE EXPERIMENT

7. Turn ON the PBR FMT150 main switch on the front panel.
8. Turn ON the PC and start the PBR client as described in PBR Software manual.
9. Connect the pH/temperature sensor with the PBR FMT150.
10. Before starting new experiment it is recommended to perform pH sensor calibration with commercial pH standards as described on page 27 or in PBR Software manual. Perform two point calibration with standard pH 7 and pH 10.

Optionally: pH calibration can be performed also prior to the vessel autoclaving step. Sensor can be thus autoclaved already inserted in the cultivation vessel.

11. Disconnect the pH/temperature sensor and place it together with the autoclaved cultivation vessel inside a sterile flow hood.
12. Sterilize the pH electrode with hot or distilled water and mount it immediately in the autoclaved vessel. Other options for sterilization: use 0.1 M HCl or ethanol rinse.
13. Add medium to the cultivation vessel through the open port for additional probe using sterilized funnel.

Optional: If using magnetic stirrer, insert the sterilized magnetic stirring bar into the vessel.

Optional: Install other sterilized sensors such as O₂ and CO₂ sensor if required.

14. Close the remaining open vessel ports with autoclaved screws.
15. Connect all tubing for gas inlet and gas/medium outlet and sampling as described in Fig. 13.

6.Cultivation Guide

16. Close all openings, except the one for medium outlet, using sterile connectors.
17. Take the assembled vessel out of the hood and place it in the PBR, lock it with the side clips. Connect the pH/temperature probe with the PBR (as described on page 26).
18. **Note:** It is recommended to let the medium in the vessel reach the desired cultivation temperature before proceeding with the next step (calibration).
19. Prior to start of each experiment OD calibration should be performed with the medium used in that experiment.

Important Note: It is important to cover the front side of the vessel with the dark LED panel shield (provided with the system) for the OD calibration to avoid possible interference of external light.

OD calibration can be performed manually on the front panel of the FMT150 control unit or by PBR software. Please refer to chapter 8.1 on page 59 for manual control or to the PBR Software manual for calibration via software.

20. After performing OD calibration add the prepared inoculum solution up to the desired OD value to start the cultivation. Perform this step in the sterile environment of the flow hood.

Optional: User can employ a peristaltic pump to gradually introduce the inoculum into the vessel while monitoring OD of the culture until the desired value is reached for optimal culture initiation.

21. Return the vessel with the culture to the photobioreactor.
22. Connect the vessel with the air line as described on page 29 (Fig. 18).
23. **Note:** If optional accessories are used follow the instructions for their installation.
24. Configure the experimental protocol in the PBR software and start the experiment. Detail description of these steps are available in PBR Software manual.

Note: When using the photobioreactor without the PBR software, follow the manual instructions for the setup of the growth parameters using the front panel of the PBR FMT150 control unit and as described in chapter 8.1 on page 59.

7. TROUBLESHOOTING AND CUSTOMER SUPPORT

In case of troubles and for customer support, please, write to support@psi.cz or contact your local distributor.

8. APPENDIX

8.1 FMT150 CONTROL MENU TREE

This section explains the operation of the Photobioreactor FMT150 control. Photobioreactor can operate in cultivation mode by setting the cultivations parameters such as light, temperature, etc. in the menu navigation tree. Please note that even though various parameters such as chlorophyll fluorescence and OD, temperature, etc can be measured, the values and data are not recorded and are only displayed at the time of the measurement. The operation of the PBR control unit is structured into four levels:

1. Main Menu
2. Four Sub-Menus (Measure, Lights, Thermoregulation, Setting)
3. First-level nested structures
4. Second-level nested structures

The following instructions describe operation of the PBR FMT150 in cultivation mode only.

8.1.1 CONTROL KEYS AND USED SYMBOLS

The Photobioreactor is operated by five keys:

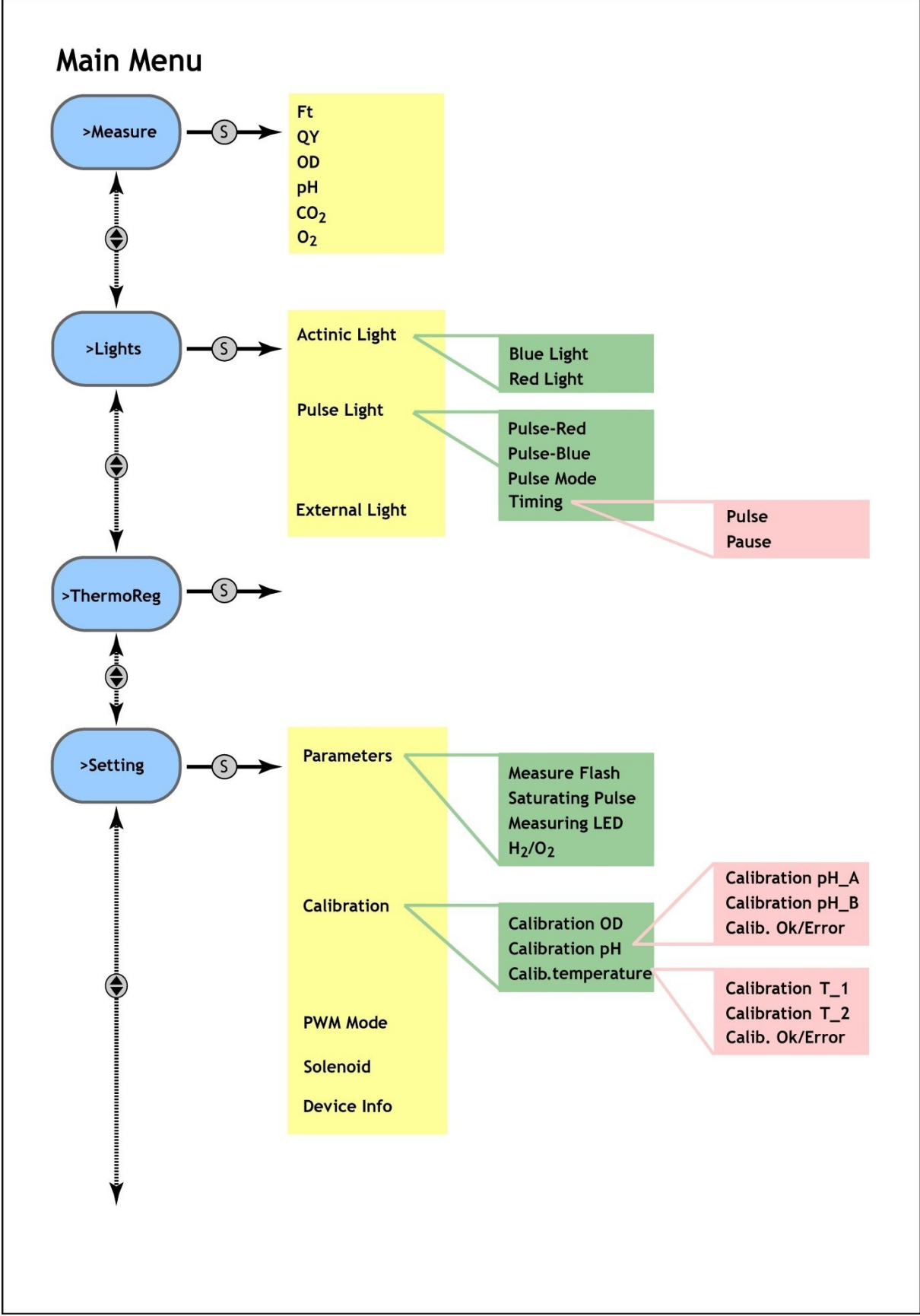
- The **“MAINS”** key is intended only for service purposes and doesn't have any function related to the common use of PBR. The Photobioreactor is turned on by switching on the corresponding Power supply.
- The **“S”** key allows to select a submenu and it is also used as a “Confirm Selection” key. Long pressed **“S”** key (= **“HS”**) is mostly used for moving inside the single menus (scrolling).
- The **“M”** key allows moving back to a parent menu.
- The two **“Scroll”** keys allow moving up and down inside the single menu.

Explanation of symbols and colour differentiation used in the schematic:*

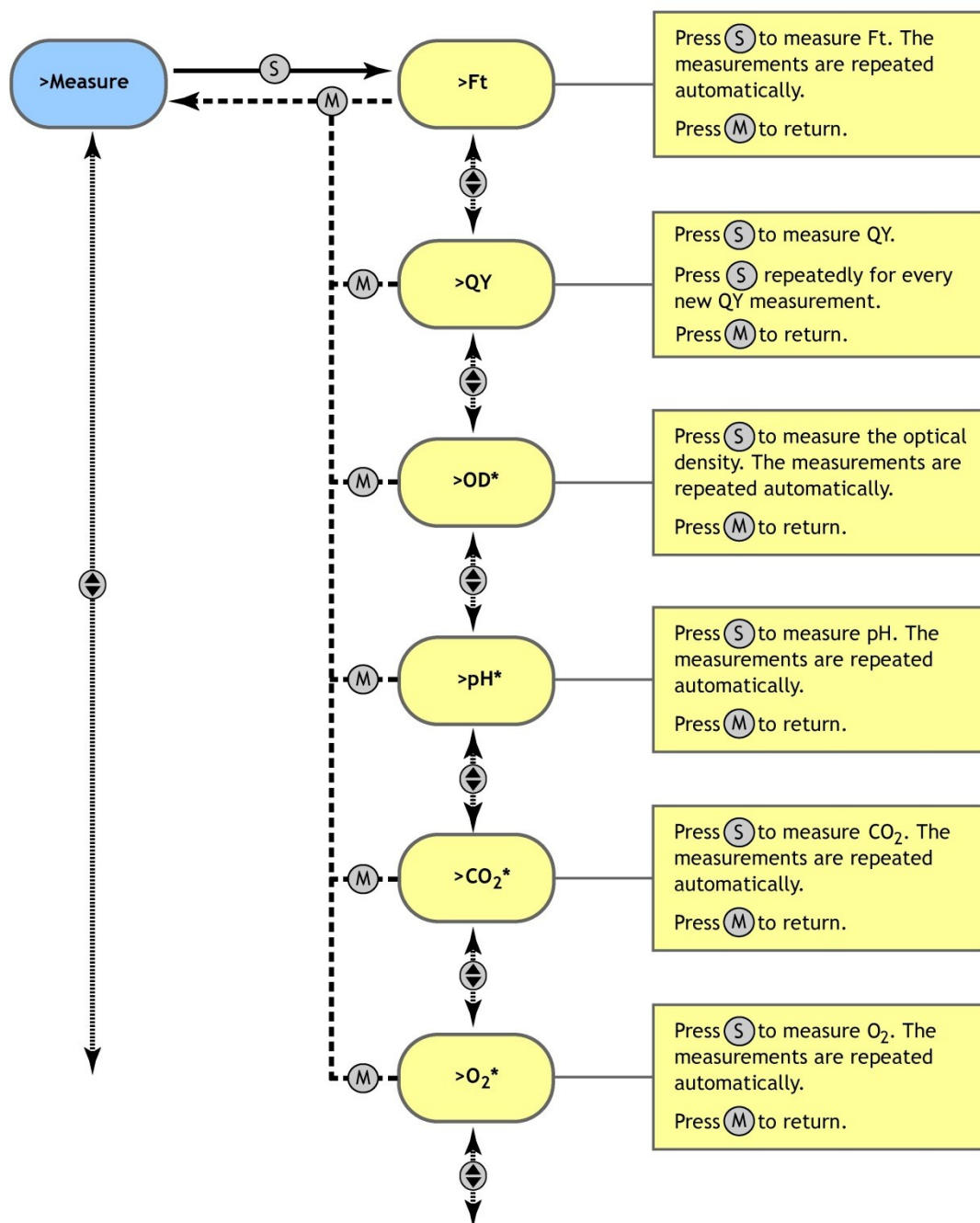
- **SET key (short press)**
- **MENU key (short press)**
- **UP/DOWN key**
- **Hold SET key = SET key (long press)**
- **SET key (short press or long press)**
- **Hold MENU key = MENU key (long press)**
- **MENU key = (short press or long press)**
- Full-line arrows are used for the **“S”** key.
- Dashed-line arrows are used for the **“M”** key.
- Dotted-line arrows are used for the **“UP/DOWN”** key.
- **Blue** colour represents the **Main Menu**.
- **Yellow** colour represents the **Sub-Menu** items.
- **Green** colour corresponds to the **first-level nested structures**.
- **Pink** colour corresponds to the **second-level nested structures**.

* The display of the Photobioreactor does not reflect this colour differentiation.

8.1.2 OPERATION SCHEMATIC



Measure Sub-Menu



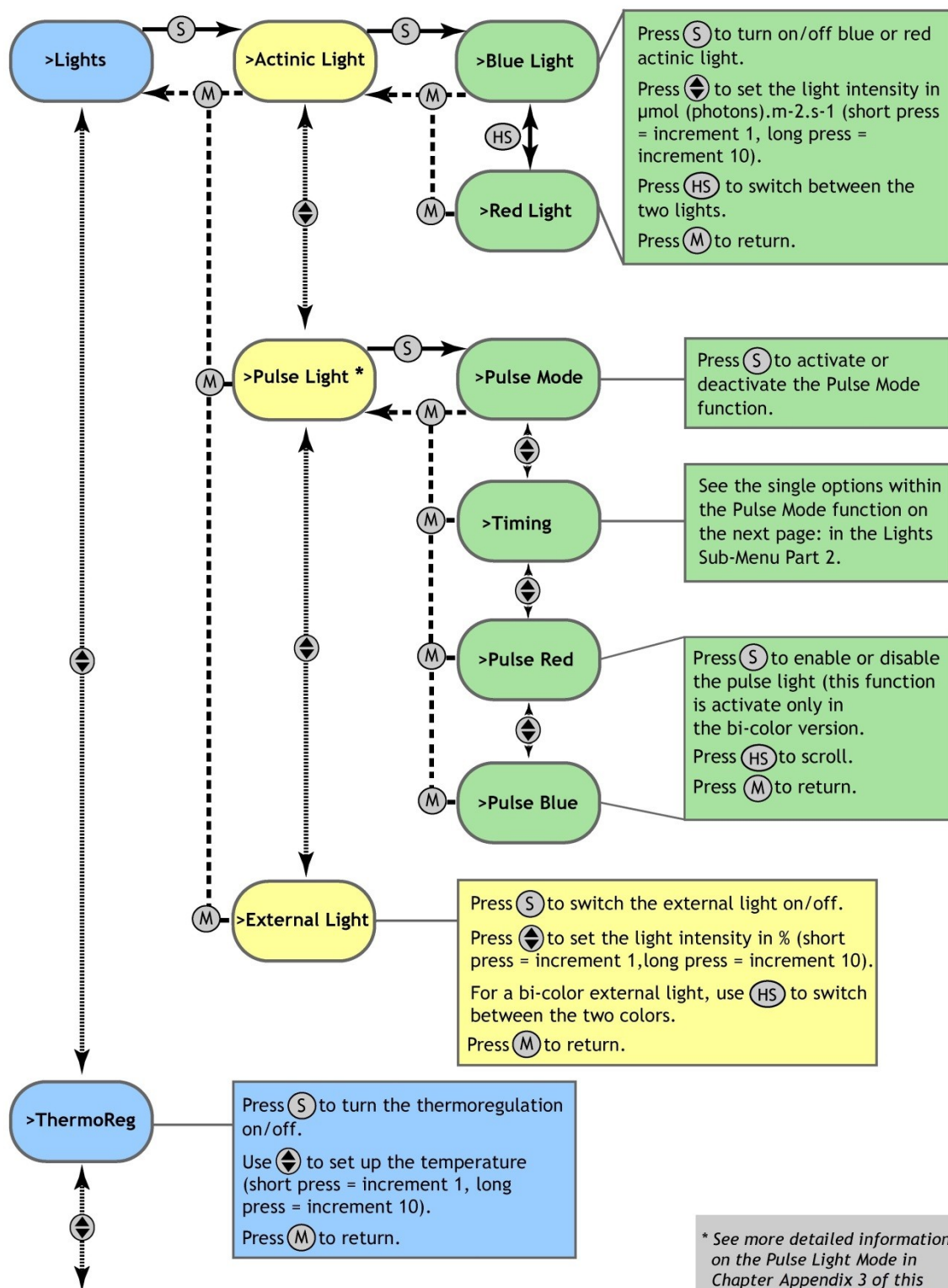
***Abbreviations explanation:**

[CAL] = OD / pH calibrated

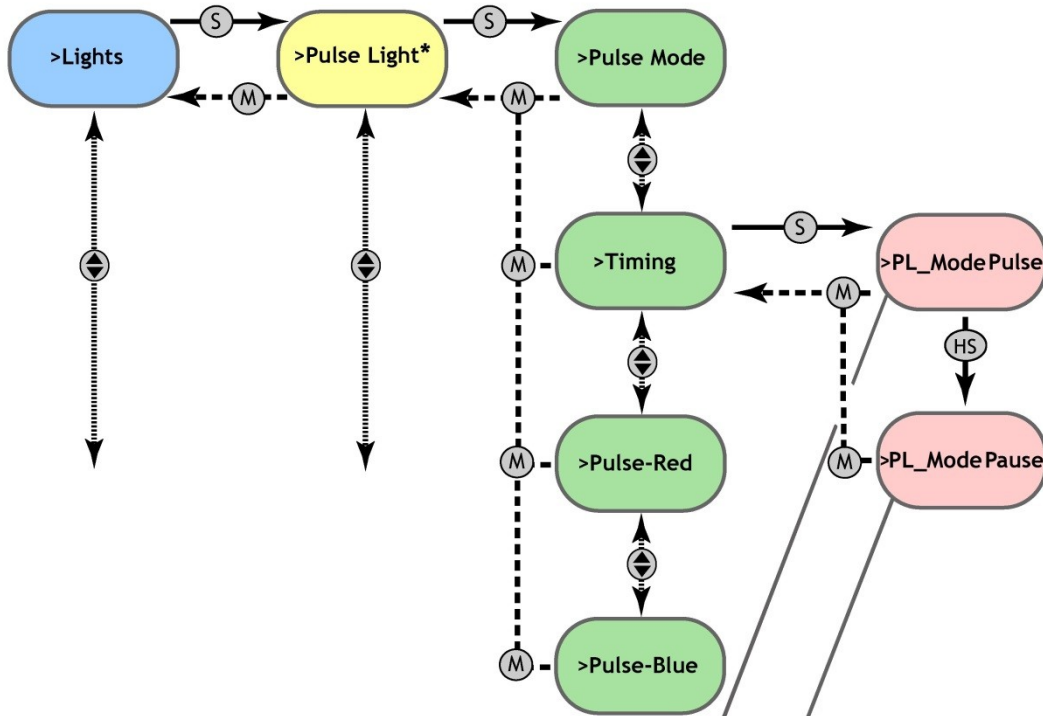
[NCL] = OD / pH not calibrated

[Dis] = CO₂/ O₂ module disabled

Lights and Thermoregulation Sub-Menu



Lights Sub-Menu (Part 2)



Press (S) for setting the pulse time interval (pulse width).

Press (⬆) to set the pulse time interval in format: SECOND :
MILLISECOND : MICROSECOND (second <0;3600>; millisecond <0;999>;
microsecond <0;900>).

Press (HS) to scroll. Press (M) to return.

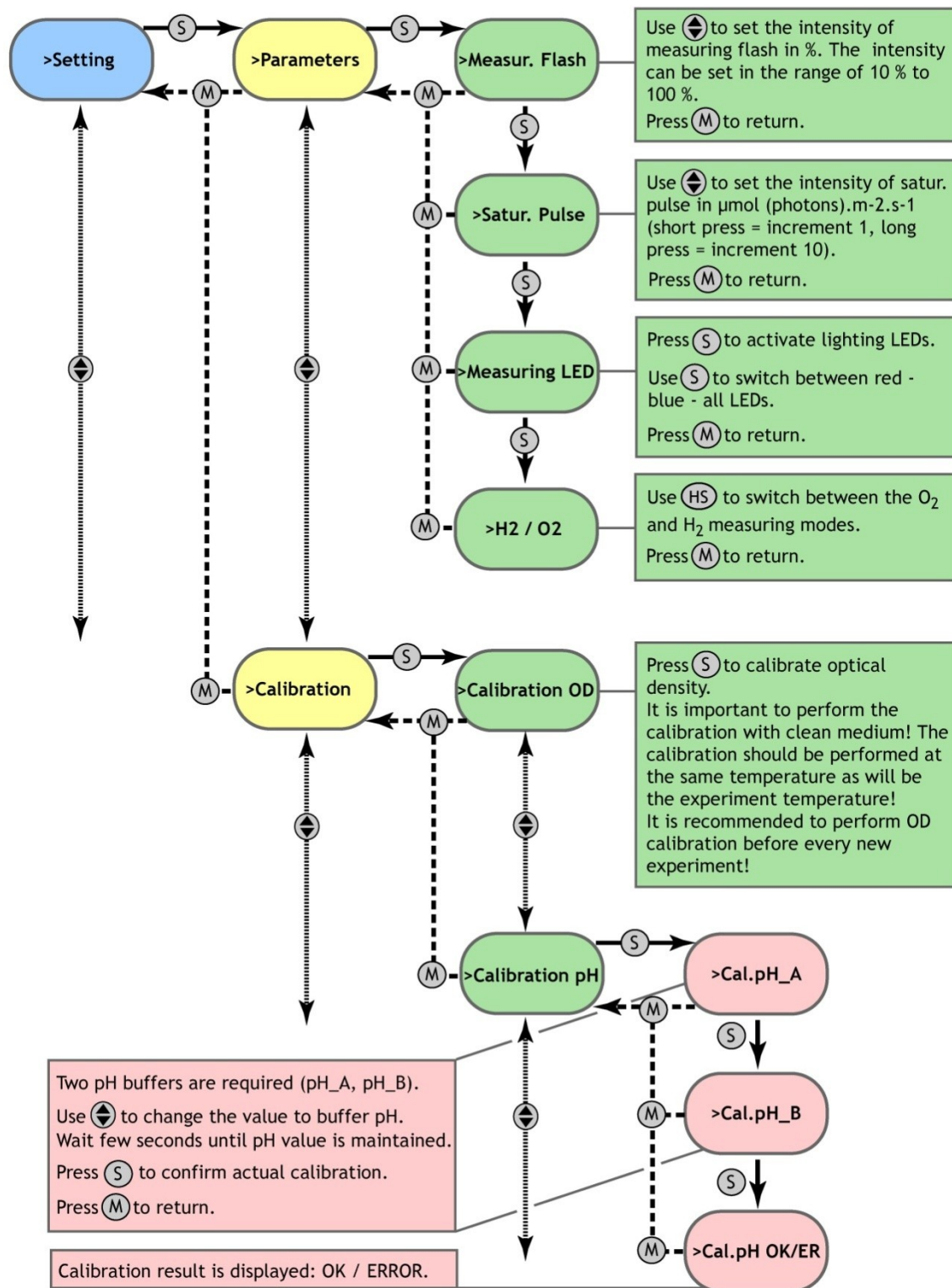
Press (S) for setting the pause time interval (pause width).

Press (⬆) to set the pulse time interval in format: SECOND :
MILLISECOND : MICROSECOND (second <0;3600>; millisecond <0;999>;
microsecond <0;900>).

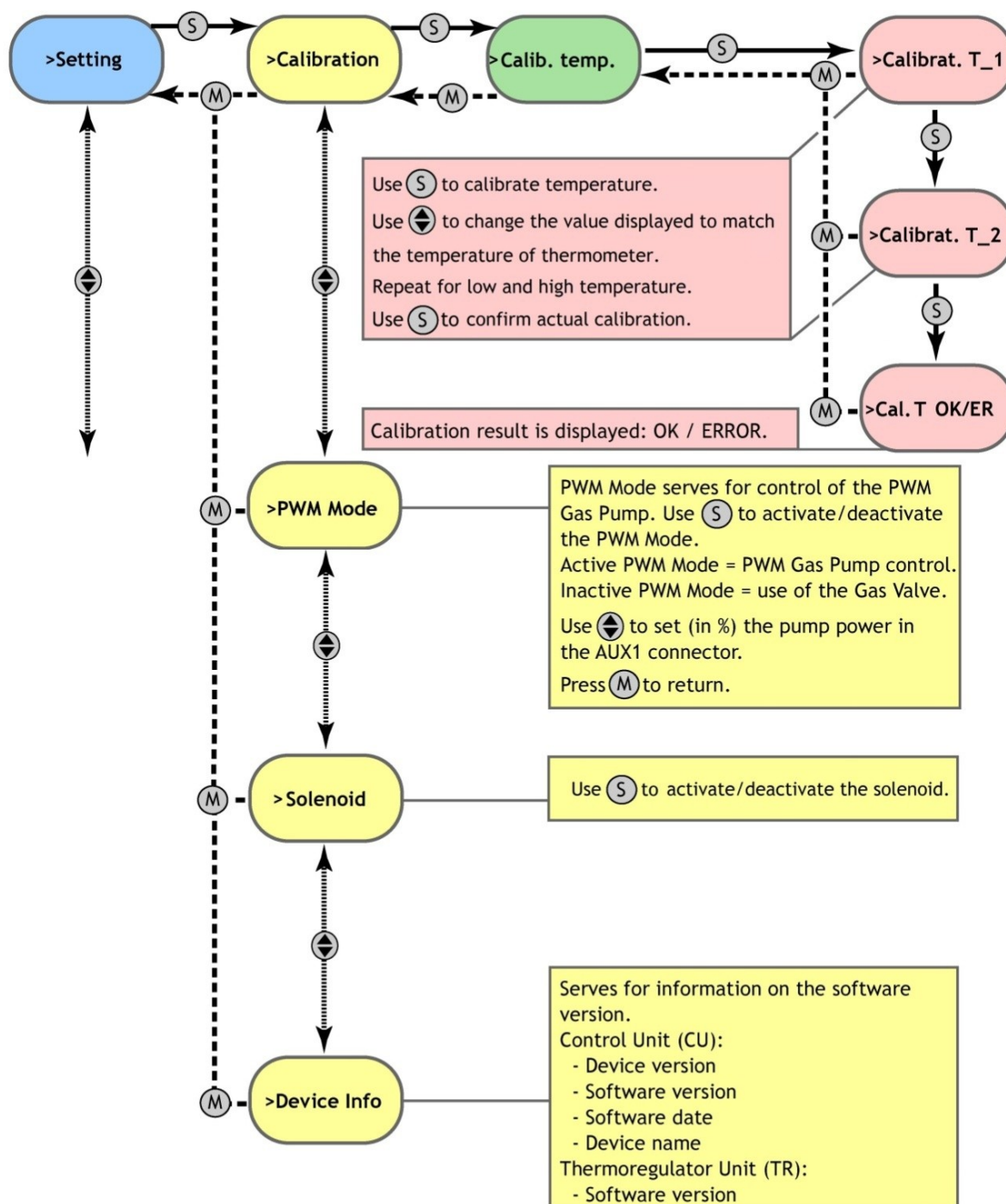
Press (HS) to scroll. Press (M) to return.

* See more detailed information
on the Pulse Light Mode in
Chapter Appendix 3 of this
Manual.

Setting Sub-Menu (Part 1)



Setting Sub-Menu (Part 2)



8.1.3 PULSE LIGHT CONTROL

Pulse light control mode is designed to study the response of the cultivated culture to rapidly repeated flashes of actinic lights (red, blue or white combination depending on purchased combination of actinic lights).

Control keys used are described in detail in Appendix on page 59.

Note: Please note that the Pulse Light Control protocol can be operated only via front panel of the PBR FMT150 control unit and is currently not supported in PBR software.

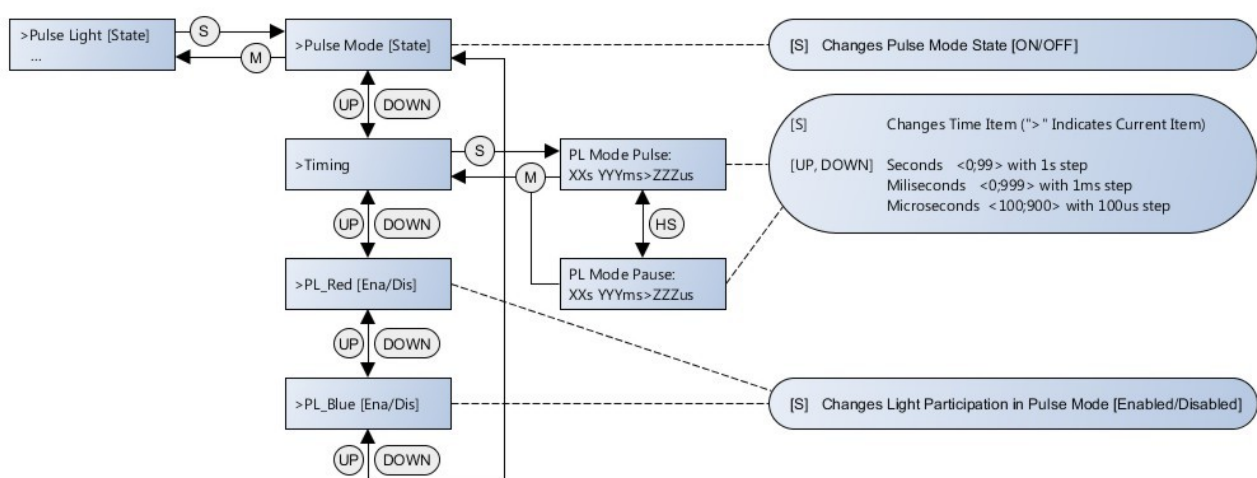


Fig. 46: Pulse light menus

8.2 QY MEASUREMENT

Below is shown schematic description of the QY measurement.

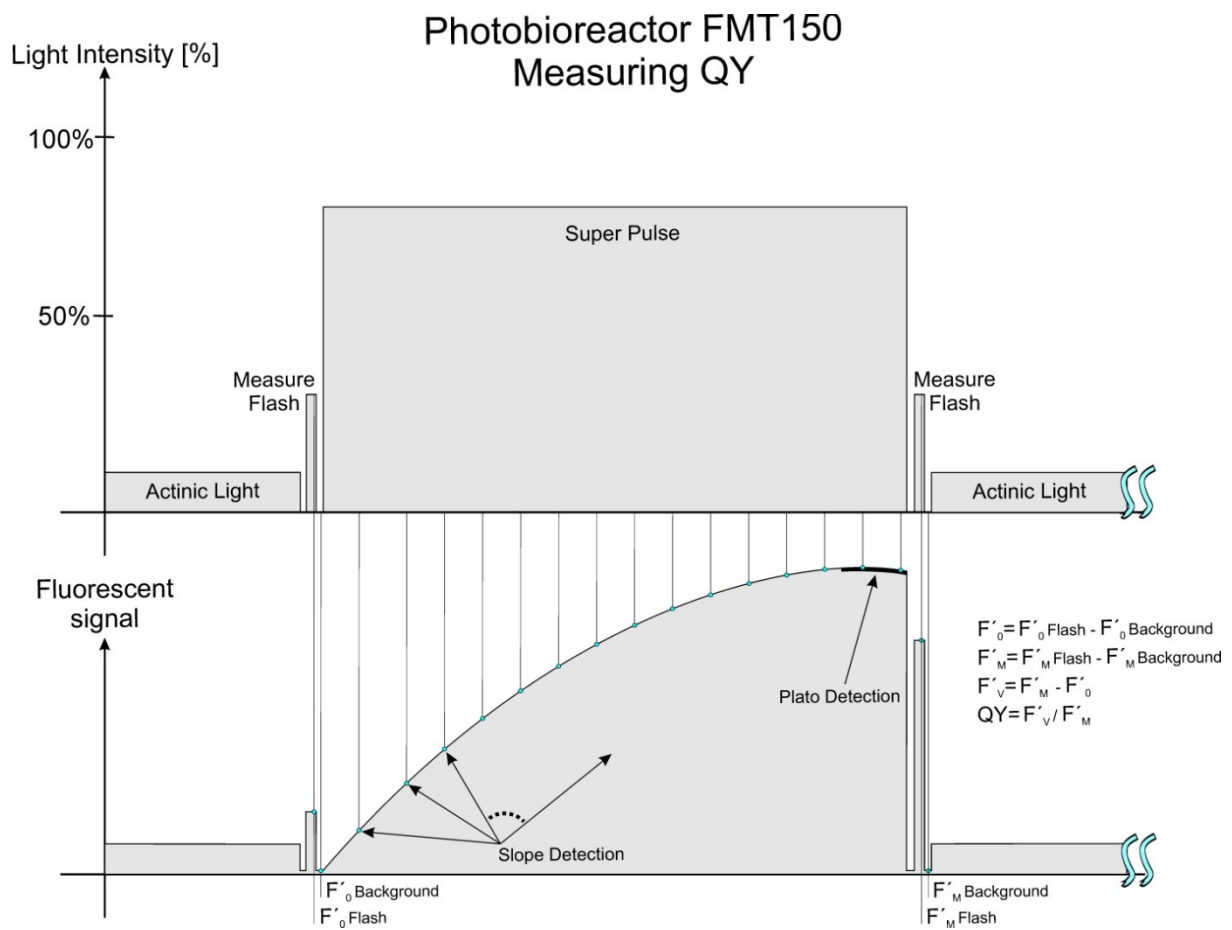


Fig. 47: QY measurement theory

8.3 TECHNICAL SPECIFICATION STANDARDS

8.3.1 FMT150 400ML

Measured chlorophyll Fluorescence Parameters:

F_o, F_t, F_m, F_m' , F_v/F_m. Other parameters can be calculated.

Vessel Capacity / Weight:

400 ml / 2.8 kg

Precision Controlled Temperature:

Standard: 15 °C - 55 °C

Optional: 10 – 70 °C

Temperature Sensor:

Platinum Pt 1,000 in thermo-well

Cooling and heating system:

200 W Peltier elements

Two Displays:

System control and actual temperature

Actinic and Saturating Light:

Two standard versions: one with blue and red LEDs (450 nm, 630 nm); the other with white and red LEDs. Light intensity is adjustable from 0 ~ 1500 μmol(photon)/m².s, with light upgrade 0 ~ 3000 μmol(photon)/m².s. Other LED colors available as an option.

OD - Optical Density Measurement:

Real time measurement of OD by two LEDs (735 nm for version FMT150.1, 720 nm for version FMT150.2, 680 nm for both FMT 150.1 and FMT150.2)

Detector Wavelength Range:

PIN photodiode with 665 nm - 750 nm bandpass filters

Controlled Flow and Composition of the Bubbling Air:

Optional

Pumps:

Up to 8 peristaltic pumps (optional)

Probes (pH, O₂, CO₂):

Optional

BIOS:

Upgradeable firmware

Communication Port:

Serial - RS232 for FMT150.1

USB port for FMT150.2

Cultivation Vessel Material:

Glass, stainless steel, duralumin, silicone sealing

Size:

41,5 x 35 x 31 cm

Weight:

15.5 kg

Electrical:

90-240 V AC

8.3.2 FMT150 1000ML

Measured chlorophyll Fluorescence Parameters:

F_o, F_t, F_m, F_m' , F_v/F_m. Other parameters can be calculated.

Vessel Capacity / Weight:

1000 ml / 3.8 kg

Precision Controlled Temperature:

Standard: 15 °C - 55 °C

Optional: 10 – 70 °C

Temperature Sensor:

Platinum Pt 1,000 in thermo-well

Cooling and heating system:

200 W Peltier elements

Two Displays:

System control and actual temperature

Actinic and Saturating Light:

Two standard versions: one with blue and red LEDs (450 nm, 630 nm); the other with white and red LEDs. Light intensity is adjustable from 0 ~ 1500 μmol(photon)/m².s, with light upgrade 0 ~ 3000 μmol(photon)/m².s. Other LED colors available as an option.

OD - Optical Density Measurement:

Real time measurement of OD by two LEDs (735 nm for version FMT150.1, 720 nm for version FMT150.2, 680 nm for both FMT 150.1 and FMT150.2)

Detector Wavelength Range:

PIN photodiode with 665 nm - 750 nm bandpass filters

Controlled Flow and Composition of the Bubbling Air:

Optional

Pumps:

Up to 8 peristaltic pumps (optional)

Probes (pH, O₂, CO₂):

Optional

BIOS:

Upgradeable firmware

Communication Port:

Serial - RS232 for FMT150.1

USB port for FMT150.2

Cultivation Vessel Material:

Glass, stainless steel, duralumin, silicone sealing

Size:

41,5 x 35 x 31 cm

Weight:

17.5 kg

Electrical:

90-240 V AC

8.3.3 FMT150 3000ML

Measured chlorophyll Fluorescence Parameters:

F_o, F_t, F_m, F_m' , F_v/F_m. Other parameters can be calculated.

Vessel Capacity / Weight:

3000 ml / 6.2 kg

Precision Controlled Temperature:

Standard: 15 °C - 55 °C

Optional: 10 – 70 °C

Temperature Sensor:

Platinum Pt 1,000 in thermo-well

Cooling and heating system:

400 W Peltier elements

Two Displays:

System control and actual temperature

Actinic and Saturating Light:

Two standard versions: one with blue and red LEDs (450 nm, 630 nm); the other with white and red LEDs. Light intensity is adjustable from 0 ~ 1500 μmol(photon)/m².s, with light upgrade 0 ~ 3000 μmol(photon)/m².s. Other LED colors available as an option.

OD - Optical Density Measurement:

Real time measurement of OD by two IR LEDs (720 nm, 680 nm)

Detector Wavelength Range:

PIN photodiode with 665 nm - 750 nm bandpass filters

Controlled Flow and Composition of the Bubbling Air:

Optional

Pumps:

Up to 8 peristaltic pumps (optional)

Probes (pH, O₂, CO₂):

Optional

BIOS:

Upgradeable firmware

Communication Port:

USB port for FMT150.2

Cultivation Vessel Material:

Glass, stainless steel, duralumin, silicone sealing

Size:

50 x 35 x 31 cm

Weight:

28 kg

Electrical:

90-240 V AC

8. Appendix

8.3.4 GAS 150

Warm-up time: 45 minutes

Input/Output Connector: Parker Prestolock 6 mm

Weight: 5 kg

Dimensions: 41x37x17 cm

Carbon Dioxide Module – Specification:

Available CO₂ ranges: 0-2 %, 0-3 %, 0-5 %, 0-10 %, and 0-20 % (please note that if any other than standard range is required, the customer needs to specify the required range)

Standard Range: 0-2 %

Accuracy at +25 °C (+77 °F) against certified factory references: $\leq \pm[0.02\% \text{ CO}_2 + 2\% \text{ of reading}]$

Nonlinearity: $\leq \pm 1.0\% \text{ FS}$

Temperature dependence of output: (typ.) 0.1 %FS/°C (0.1 %FS/°F)

Pressure dependence: typically 0.15% of reading/hPa

Long-term stability: $\leq \pm 5\% \text{ FS}/2 \text{ years}$

Response time (63 %): 20 seconds

Flow meter/controller - Specification:

Range: based on customer's specifications

Default range: 1 l/min (0.02- 1 l/min)

Accuracy (incl. linearity): $\pm 1\% \text{ FS}$ plus $\pm 1\% \text{ RD}$

Repeatability: $< 0.2\% \text{ FS}$ typical

Settling time: approx. 2 seconds

Temperature sensitivity: zero: $< 0.1\% \text{ FS}/^\circ\text{C}$; span: $< 0.2\% \text{ RD}/^\circ\text{C}$

Pressure sensitivity: 0.3% RD/bar typical at Air

Attitude sensitivity: max. error at 90° off horizontal 0.2 % at 1 bar

Input Pressure: 3 bar

9. WARRANTY TERMS AND CONDITIONS

- This Limited Warranty applies only to the Photobioreactor FMT150. It is valid for one year from the date of shipment.
- If at any time within this warranty period the instrument does not function as warranted, return it and the manufacturer will repair or replace it at no charge. The customer is responsible for shipping and insurance charges (for the full product value) to PSI. The manufacturer is responsible for shipping and insurance on return of the instrument to the customer.
- No warranty will apply to any instrument that has been (i) modified, altered, or repaired by persons unauthorized by the manufacturer; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by the manufacturer.
- The warranty is return-to-base only, and does not include on-site repair charges such as labor, travel, or other expenses associated with the repair or installation of replacement parts at the customer's site.
- The manufacturer repairs or replaces faulty instruments as quickly as possible; the maximum time is one month.
- The manufacturer will keep spare parts or their adequate substitutes for a period of at least five years.
- Returned instruments must be packaged sufficiently so as not to assume any transit damage. If damage is caused due to insufficient packaging, the instrument will be treated as an out-of-warranty repair and charged as such.
- PSI also offers out-of-warranty repairs. These are usually returned to the customer on a cash-on-delivery basis.
- *WEAR & TEAR ITEMS* (such as sealing, tubing, padding, etc.) are excluded from this warranty. The term *WEAR & TEAR* denotes the damage that naturally and inevitably occurs as a result of normal use or aging even when an item is used competently and with care and proper maintenance.

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10. LIST OF ABBREVIATIONS

E – Actinic Illumination Irradiance

F_T, F'₀ – continuous fluorescence level

F₀ - continuous fluorescence level of dark adapted sample

F_M – maximal fluorescence level

F'_M – maximal fluorescence level in light-adapted sample

F_v - variable fluorescence parameter $F_v = F_M - F_0$

F'_v - variable fluorescence parameter $F'_v = F'_M - F_T$

LCD – Liquid Crystal Display

LED – Light Emitting Diode

OD680 – Optical Density at 680 nm

OD720 – Optical Density at 720 nm

PAR – Photosynthetically Active Radiation

PBR – Photobioreactor

PSII – Photosystem II

P_e – Photosynthetic electron transport rate

QY – Photosystem II Quantum Yield