

RTS-8

Personal Multi-channel Bioreactor



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1. About this edition of the instructions

- 1.1 The current edition of the user instructions applies to the following versions of personal multi-channel bioreactor:
- RTS-8 version V.1A01

2. Safety Precautions



Caution! Make sure you have fully read and understood the present Manual before using the equipment. Please pay special attention to sections marked by this symbol.



Caution! Surfaces can become hot during use.

GENERAL SAFETY

- Use only as specified in the operating manual provided. Safety of use of the product may be impaired if it is used not in the indicated manner, or if accessories (falcon tubes) are used that do not match the required characteristics
- The unit should not be used if dropped or damaged.
- After transportation or storage, keep the unit under room temperature for 2 - 3 h before connecting it to the electric circuit.
- Store and transport the unit at ambient temperatures between -20°C and +60°C and maximum relative humidity of 80%.
- Before using any cleaning or decontamination methods except those recommended by the manufacturer, check with the manufacturer that the proposed method will not damage the equipment.
- Do not make modifications in design of the unit.
- The device is optimized to work only with falcon 50 ml tubes, all other ways of applying the unit is forbidden.



Caution! The unit is heavy (20 kg). It is required to lift the unit only by holding it firmly with both hands under the left and right sidewall recesses.

ELECTRICAL SAFETY

- Do not plug the unit into the main socket without grounding, and do not use extension lead without grounding.
- Connect only to a power supply with voltage corresponding to that on the serial number label.
- Disconnect the unit from the electric circuit before moving.
- Turn off the unit by switching off the power switch and disconnecting the external power supply from the power socket.
- Ensure that the power switch on the rear side of the unit and the power plug are easily accessible during use.
- This unit is controlled by PC. Please ensure that the attached PC itself conforms to safety and EMC standards.
- If liquid penetrates into the unit, disconnect it from the external power supply and have it checked by a repair and maintenance technician.
- Do not operate the unit in premises where condensation can form. Operating conditions of the unit are defined in the **Specifications** section.

DURING OPERATION

- Do not operate the unit in environments with aggressive or explosive chemical mixtures. Please contact manufacturer for possible operation of the unit in specific atmospheres.
- During installation, ensure gaps of at least 15 cm from the walls of the unit to other items to ensure normal operation (in particular, to ensure adequate ventilation).
- Do not operate the unit if it is faulty or has been installed incorrectly.
- Do not use outside laboratory rooms.
- Do not check the temperature by touch. Use a thermometer.
- Always clean and decontaminate the socket and the lid after operation.
- Take care when operating near the rotating tube sockets

BIOLOGICAL AND CHEMICAL SAFETY

- During the mechanical and heat treatment of materials, the formation of dangerous gases and substances (including flammable) is possible and care must be taken.
- It is the user's responsibility to carry out appropriate decontamination if hazardous material is spilt on or penetrates into the equipment. Means for disinfection should be such that there are no hazardous chemical reactions between spilled materials and cleaning agents. If necessary, consult the manufacturer.
- The tube of the bioreactor must be sealed very tightly. Please see **4.5** for instructions on testing the tubes.



Caution! The product is not intended for use in hazardous environments and with hazardous materials (chemically active / aggressive, explosive, etc.). Do not mix flammable liquids if this can lead to danger.

3. General Information

RTS-8 is a personal bioreactor that utilizes patented Reverse-Spin® technology that applies non-invasive, mechanically driven, low energy consumption, innovative type of agitation where cell suspension is mixed by the single-use falcon bioreactor tube rotation around its axis with a change of direction of rotation motion resulting in highly efficient mixing and oxygenation for aerobic cultivation. Combined with a near-infrared optical system it is possible to register cell growth kinetics noninvasively in real time.

The Personal Bioreactor is applicable in:

- Microbiology
- Molecular biology
- Cell biology
- Biotechnology
- Biochemistry
- Systems Biology
- Synthetic Biology

Typical applications:

- Fermentation real time growth kinetics
- Clone candidate screening
- Protein expression
- Temperature stress and fluctuation experiments
- Media screening and optimization
- Growth characterization
- Inhibition and toxicity tests
- Strain quality control

Features:

- Parallel cultivation enables to save time and resources for bioprocess optimization
- Individually controlled bioreactor accelerates optimization process
- Possibility to cultivate microaerophilic and obligate anaerobic microorganisms (not strict anaerobic conditions)
- Reverse–Spin® mixing principle enables non-invasive biomass measurement in real time
- Near-infrared optical system makes it possible to register cell growth kinetics
- Free of charge software for storage, demonstration and analysis of data in real time
- Compact design with low profile and small footprint for personal application
- Individual temperature control for bioprocess applications
- Active cooling for rapid temperature control, e.g. for temperature fluctuation experiments
- Task profiling for process automatization
- Cloud data storage to monitor the process of cultivation while away or using a smartphone

To fully use RTS-8 capabilities, the device must be connected to a PC and RTS-8 software. The device cannot be used as a standalone unit. Software possibilities:

- Real-Time cell growth logging
- 3D graphical representation of OD or growth rate over time over unit
- Pause option
- Save/Load option
- Report option: PDF and Excel
- Connect up to 3 units (recommended) simultaneously to 1 computer
- Remote monitoring option (requires internet connection)
- Cycling/Profiling options
- User manual calibration possibility for most cells.

4. Getting started

4.1 **Unpacking.** Remove packing materials carefully and retain them for future shipment and storage of the unit. Examine the unit carefully for any damage incurred during transit. The warranty does not cover in-transit damage.

4.2 **Complete set.** The unit set includes:

- RTS-8, Multi-channel bioreactor..... 1 pce
- Blackout lids with ventilation..... 8 pcs
- Bioreactor vessels TPP TubeSpin® Bioreactor 50ml..... 20 pcs
- USB data cable 1 pce
- USB disk drive with software installation files and manual..... 1 pce
- Power cable 1 pce
- Operating Manual, Certificate..... 1 copy

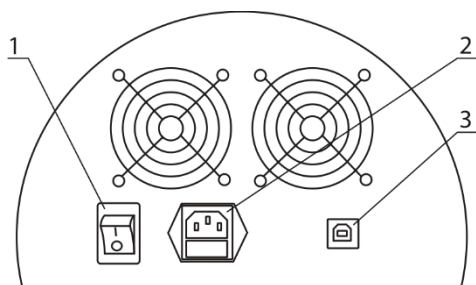


Figure 1. Rear panel of the unit

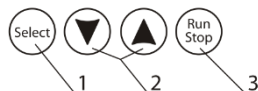
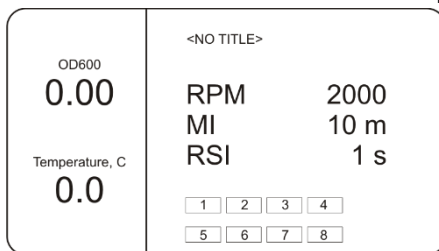


Figure 2. Control panel

4.3 **Setup.**

- Place the unit on even, horizontal working surface;
- Connect the power cable to the socket on the rear side of the unit (fig. 1/2);
- Switch on the computer, if it was turned off;
- Connect the USB data cable to the port on the rear side of the unit (fig. 1/3) and to the personal computer;
- Insert the USB disk drive in the personal computer and install the software following the software installation procedure described in software installation manual.

4.4 **Bioreactor vessel features:**

- Falcon type tubes. TPP TubeSpin® Bioreactor;
- Possible working volume 3 – 50 ml (optical system works from 7.5 to 50 ml);
- Conical form;
- 5 openings (A, B, C, D, E) of different size above the gas permeable, sterile PTFE filter of the screw cap;
- Openings can be sealed and by this, exchange adjusted to need;
- Sterile gas exchange is guaranteed by the 0.22 µm filter membrane;
- Even with a high cell density the supply of oxygen through the openings is sufficient;
- Tube fits in a standard 50 ml centrifuge rotor.

4.5 Due to the specificity of mould type manufacturing of centrifugal falcon tubes, the helical structure of the screw caps screw thread can vary, and, given the vigorous mixing conditions, the liquid can spill if the tube is not closed tightly. Tubes can be faulty and the liquid spillage is possible approximately 1 out of 60 tubes.



Before launching the experiment and leaving the device, tubes must be checked for liquid spillage occurring in a period of at least 2 minutes at 2000 RPM and 1 s^{-1} Reverse Spin Interval (RSI) with a closed lid. If droplets of liquid will appear on the inner surface of the lid, then the screw cap is faulty and the tube must be replaced.

4.6 **Change of optical characteristics of the tube depending on temperature:**
When temperature of the plastic material is changing, i.e. during temperature change of 30°C every hour, the plastic material of the tube changes optical characteristics in a range of $\pm 0.1 \text{ OD}_{600}$.

5. Calibration

5.1 **Calibration verification.** The device is software calibrated with *E.coli* BL21 or *S.Cerevisiae* wild strain cell suspensions for operation with TPP TubeSpin® Bioreactor 50ml tube at temperature range from +15°C to +60°C.

To verify the conformity of calibration follow the subsequent procedures:

- Connect the device to the computer, launch the software and select factory calibration;
- Take a TPP TubeSpin® Bioreactor 50ml tube;
- Add 10 ± 0.1 ml distilled water;
- Close the cap of the tube thoroughly;
- Insert the tube into the socket;
- Set the measurement interval (MI) to 1 minute;
- Press the **Play** button in the software;
- The device will start measuring in 1 minute and should complete after 30—60 seconds and OD value should appear on the display and software;
- If OD value equals $0 (\pm 0.1 \text{ OD})$ then the device corresponds to factory pre-calibration settings and is suitable for use.

5.2 Creating user calibration

5.2.1 Get cell suspension samples in 50 ml falcon tubes with typical optical densities of your experiments. If the maximal OD of your experiment (stationary phase) is 5 OD₆₀₀ then the recommended samples are 0 (ddH₂O water or broth media) 1, 2, 3, 4, 5, 6 OD₆₀₀.

Measure OD at desired wavelength of each cell suspension using a spectrophotometer with proper prior dilutions. The proportionality between OD₆₀₀ and cell density exists only for $\text{OD}_{600} \leq 0.4$ (approximately), we recommend diluting samples to the range of 0.1-0.2 OD.

Multiply the dilution factor values to get the OD of the samples.

Continue to software manual page **29**.

5.2.2 RTS-8 can be calibrated to detect scattered light of any possible cell with any possible shape and size, but due to difference of light scattering in various cell suspensions, we cannot guarantee the stated measurement range in all conditions.

6. Operation

Recommendations during operation

- Remove the falcon tube from the tube socket before connecting or disconnecting the external power supply during operation.
- Start operation approximately 15 minutes after switching on the device (some time is necessary for stabilization in the working mode).
- Tube positioning in the tube socket must be as follows: The volumes mark of the TPP tube must be between and opposite to the two markings on the rotor (figure 3); this position enables the light from the laser to be transmitted without disruption by different marks presented on the tubes outer surface.

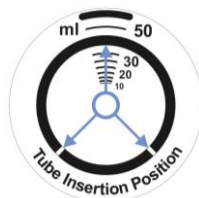


Figure 3.
Tube positioning

6.1 Connect power cable to electric circuit.

6.2 Turn on the unit by pressing the power switch on the rear panel (fig. 1/1).



Note. After turning on the unit starts heating and continues to maintain the temperature regardless of other operations.

6.3 Insert the tube into the sockets.

6.4 **Software control mode.** Switch on the computer with installed software and continue working according to software operation manual.



Note. While the unit is controlled by PC, front panel keys are limited in functions, only **Run Stop key** functions. The display of the unit shows “RTS-8 controlled by PC”.

6.5 **Manual mode.**

6.5.1 Press the **Select** key (fig. 2/1) to activate the possibility to change to an individual channel or to a parameter (the channel box or parameter will be highlighted and blinking). Selected channel box will remain blinking all the time while the device is on. Indications of colors of boxes are the following:

- Brown when channels are not operating.
- Yellow when a channel is actively selected by **Select** key (lasting 10 seconds) which allows to switch between channels.
- Green when channels are in operation,

6.5.2 Use ▲ and ▼ keys (fig. 2/2) to change to an individual channel or set the necessary value (the box will be highlighted and blinking).

6.5.3 It is possible to set by ▲ and ▼ keys time between optical density measurements – MI, channel selection, spinning speed (RPM), temperature (°C), temperature control (on/off), Reverse Spins Interval (RSI).

6.5.4 Press the **Run Stop** key (fig. 2/3) to start and stop operation.



Caution! Operation stop will not stop the heating process. To stop heating process set temperature has to be decreased manually until “off” indication appears.

6.6 After finishing the operation, switch off the unit with the power switch (fig. 1/1).

6.7 Disconnect power cable from electric circuit.

7. Recommended methods for microorganism cultivation

- 7.1 **Facultative anaerobe** *Escherichia Coli*:
2700 rpm (vessel spinning speed),
1 s⁻¹ (RSI),
37° C (socket temperature),
7.5 ml (sample volume in testing vessel),
20 min., but not less (MI)
- 7.2 **Thermophilic aerobic** *Thermophilus sp.*:
2700 rpm,
1 s⁻¹ RSI,
60° C
15 ml
20 min MI
Evaporation rate at 60°C = 3.5 ml / 24 h (please adjust Volume parameter accordingly for measurement system to work correctly)
- 7.3 **Aerotolerant anaerobe** *L. acidophilus*:
0 rpm,
0 s⁻¹ RSI,
37° C,
45 ml,
20 min MI
- 7.4 **Yeasts** *S.Cerevisiae*:
2700 rpm,
1 s⁻¹ RSI,
30° C
7.5 ml
20 min., but no less, MI
- 7.5 **Obligate anaerobe** *B. bifidum*:
0 rpm,
0 s⁻¹ RSI,
37° C
50 ml (filled to the max.)
20 min MI
- 7.6 It is possible for the end-user to contact the manufacturer for advising or suggesting a required microorganism or strain to be tested. Please contact the R&D department of Biosan at these e-mail addresses:
science@biosan.lv,
igor@biosan.lv,
Igor Bankovsky, consulting biotechnologist on application questions.

8. Recommendations for creating personal settings for cultivation of microorganisms. Points to consider

- 8.1 **Temperature distribution specifics (psychrophiles, mesophiles, thermophiles).** The optimal growth temperatures of microorganisms are divided in three principal groups (see fig. 4):
- Psychrophiles (I) – obligate (1) and facultative (2);
 - Mesophiles (II);
 - Thermophiles (III) – thermotolerant (3), facultative (4), obligate (5) and extremophile (6).
- Thick line mark represents optimal growth temperature.

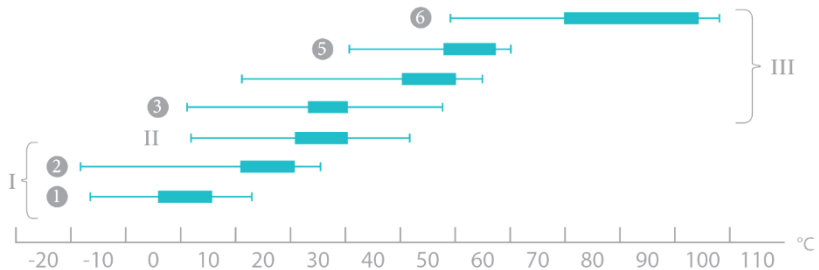


Figure 4. Temperature boarders and optimal growth zones of prokaryotes and their classification.

- 8.1.1 For psychrophiles, that are cultivated at temperatures of $15^{\circ}\text{C} \pm 2^{\circ}\text{C}$ below ambient the device must be installed in a cold room or a refrigerated chamber. Despite the active cooling of the device, the actual temperature of the reactor will always differ from the actual temperature of the sample because of its rotation.
- 8.1.2 For mesophilic microorganisms, the device can be situated at room temperature.
- 8.1.3 For thermophilic microorganisms, the device can be situated at room temperature.
- 8.2 **Cell growth depending on rotation intensity.** It is known that aeration affects the growth and growth rate of aerobic microorganisms. The RSI and RPM affect the rate of oxygen uptake in the bioreactor. Results obtained in fig. 5 and fig. 6 indicate that the maximum rate of cell division is detected at RSI of 1 s^{-1} at a speed of 2700 rpm. The increase of pause between reverse spins reduces cell growth rate and OD yield, reaching ~44% of the maximum value (RSI 1 s^{-1}), when RSI is 8 s^{-1} .
- 8.2.1 Legend of experiment (fig. 5.): Multi-channel bioreactor RTS-8 was used with 850 nm laser, volume of Terrific Broth (TB) in 50 ml Falcon tube was 10 ml, RSI 1, 2, 4, 8 s^{-1} , MI 10 min, RPM 2000, temperature 37°C , TPP Bioreactor vessels.

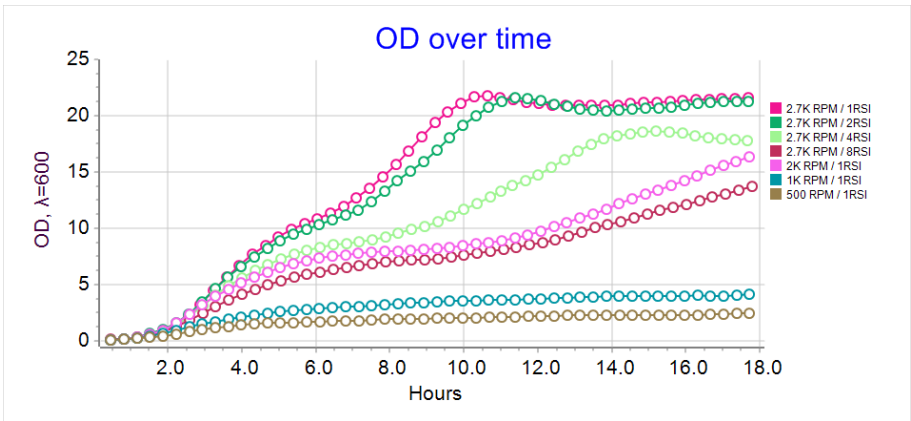


Figure 5. Influence of Interval of Reverse Spinning and RPM on the Growth kinetics ($\Delta OD_{\lambda=600nm}/\Delta t$) vs Time of fermentation (h).

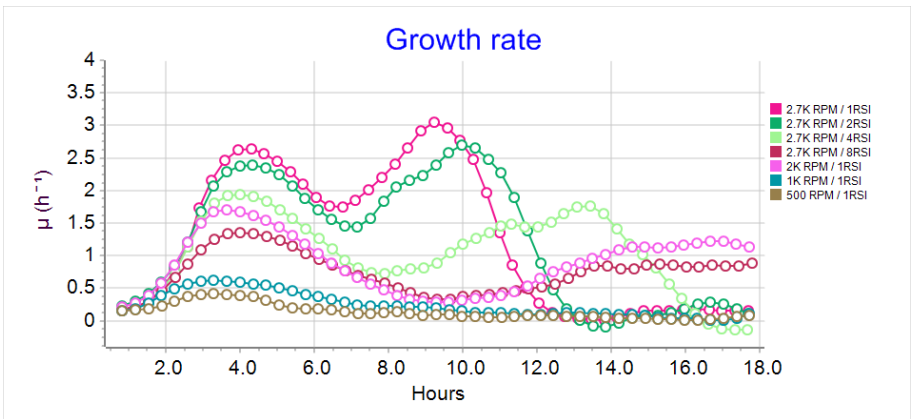


Figure 6. Influence of Interval of Reverse Spinning and RPM on the Growth kinetics ($\Delta OD_{\lambda=600nm}/\Delta t$) vs Time of fermentation (h).

8.3 Aeration and types of recommended tubes.

For aerobic microorganisms, it is recommended to use tubes that are supplied by TPP - TubeSpin® Bioreactor 50 ml. For obtaining optimal results growing aerotolerant anaerobes, it is required to seal the screw cap of TPP TubeSpin® Bioreactor 50 ml by tape or use TPP 50 ml falcon tubes that are available without air vents. User can also use standard centrifuge tubes of 50 ml Falcon type, taking into account that the tube material will be as transparent as TPP TubeSpin® Bioreactor tube or must create user calibration.

- 8.4 The cells that are used for factory calibration are *E.coli* BL21 (freshly grown using TB medium overnight) or *S.Cerevisiae* wild strain (freshly grown using YPD medium overnight).

8.5 **Factory calibration growth phase influence on achievable user calibration measurement error.**

During the growth transition of cells from the exponential growth to the stationary phase, a number of morphological and physiological changes take place, including cell volume decrease and cell shape change. Therefore, if cells are taken for referent measurement using spectrophotometer at different stages from stationary phase then the correctness of measurement can be worse than specified. Moreover, OD measurement results of spectrophotometers differ from one another and depend on the optical configuration such as aperture size for example. Therefore, it is a requirement for application of the same spectrophotometer OD measurement for results repeatability.

8.6 **User calibration.**

Calibration depends on the cell size and volume. Calibration from one type of microorganism cannot be used accurately for other type microorganism of other size and shape. The device can be calibrated at desired reference wavelength to meet the needs of the user, yet the full specified measurement range cannot be guaranteed. The factory calibrations are performed using *E.coli* BL21 (stationary phase) and *S.Cerevisiae* wild strain cells (stationary phase).

9. Specification

The unit is designed for operation at ambient temperature from +4°C to +40°C in a non-condensing temperature and maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C.

Biosan is committed to a continuous program of improvement and reserves the right to alter design and specifications of the equipment without additional notice.

9.1 Optical measurement specifications

Light source	Laser
Wavelength (λ), nm	850 \pm 15
Measurement range, OD ₆₀₀	0–100
Factory calibration measurement range, OD ₆₀₀	
<i>E. coli</i>	0–50
<i>S. Cerevisiae</i>	0–75
Achievable user calibration measurement error, OD ₆₀₀	
0.1–6	\pm 0.3
6–50	\leq 5%
50–75	\leq 10%
Real time measurement, measurement interval, min	1–60
Time setting resolution, min	1

9.2 Temperature specifications¹

Setting range, °C	+15...+60
Bottom control range point, °C	15 below ambient
Top control range point, °C	60
Setting resolution, °C	0.1
Stability, °C	\pm 0.3
Sample temperature accuracy, °C	
20 °C ... 37°C	\pm 1
< 20 °C	\pm 2
> 37 °C	\pm 2

9.3 General specifications

Tube sockets	8
Sample working volume range, ml	3–50
Sample working volume for optical system to work as specified, ml	7.5–50
Speed range, rpm	150–2700
Speed setting resolution, rpm	1
Reverse spin time setting range, sec	
150–250 rpm	0
250–300 rpm	2–60
300–2700 rpm	0–60
Display	LCD
Overall dimensions (W × D × H), mm	690×350×300
Weight ² , kg	20
Input current	AC 230 V, 50 Hz
Power consumption	3.15 A / 500 W

¹ In stable ambient temperature from 20 to 25 °C

² Accurate within \pm 10%

10. Maintenance

- 10.1 If the unit requires maintenance, disconnect the unit from the mains and contact Biosan or your local Biosan representative.
- 10.2 All maintenance and repair operations must be performed only by qualified and specially trained personnel.
- 10.3 Standard ethanol (75%) or other cleaning agents recommended for cleaning of laboratory equipment can be used for cleaning and decontamination of the unit.
- 10.4 Clean the rotor of the device from liquid droplets and possible contamination after finishing fermentation.
- 10.5 Fuse replacement. Disconnect from electric circuit. Remove the power plug from the rear side of the unit (fig. 1/2). Pull out the fuse holder by applying leverage in recess (figure 7). Remove the fuse from the holder. Check and replace with the correct fuse if necessary, **M** 3.15 A for 230 V and **M** 8.0 A for 120 V (type **M** - time lag: **M**edium).

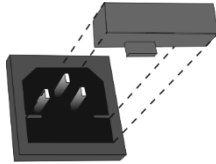


Figure 7. Fuse replacement

11. Warranty and Claims

- 11.1 The Manufacturer guarantees the compliance of the unit with the requirements of Specifications, provided the Customer follows the operation, storage and transportation instructions.
- 11.2 The warranted service life of the unit from the date of its delivery to the Customer is 24 months. For extended warranty, see 11.5.
- 11.3 Warranty covers only the units transported in the original package.
- 11.4 If any manufacturing defects are discovered by the Customer, an unsatisfactory equipment claim shall be compiled, certified and sent to the local distributor address. Please visit the **Technical support** section on our website at the link below to obtain the claim form.
- 11.5 Extended warranty. For **RTS-8**, the *Smart plus* class model, extended warranty is a paid service. Contact your local Biosan representative or our service department through the **Technical support** section on our website at the link below.
- 11.6 Description of the classes of our products is available in the **Product class description** section on our website at the link below.

Technical support



biosan.lv/en/support

Product class description



biosan.lv/classes-en

- 11.7 The following information will be required in the event that warranty or post-warranty service comes necessary. Complete the table below and retain for your records.

Model	Serial number	Date of sale
RTS-8 , Personal multi-channel bio-reactor with non-invasive real time OD measurement		

- 11.8 **Production date.** Production date is placed in the serial number, on the label of the unit. Serial number consists of 14 digits styled XXXXXXYYMMZZZZ, where XXXXXX is model code, YY and MM – year and month of production, ZZZZ – unit number.

12. EU Declaration of Conformity

- 12.1 Personal multi-channel bioreactor with non-invasive real time OD measurement
RTS-8 is in conformity with the following relevant Union legislations:

LVD 2014/35/EU	LVS EN 61010-1:2011 Safety requirements for electrical equipment for measurement, control, and laboratory use. General requirements.
EMC 2014/30/EU	LVS EN 61326-1:2013 Electrical equipment for measurement, control and laboratory use. EMC requirements. General requirements.
RoHS3 2015/863/EU	Directive on the restriction of the use of certain hazardous substances in electrical and electronic equipment.
WEEE 2012/19/EU	Directive on waste electrical and electronic equipment.

- 12.2 Declaration of Conformity is available for download on the page for the relevant model on our website by links below, in the **Downloads** section:



[RTS-8](#)

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