Medical–Biological
Research & Technologies

RTS-8 plus

Personal Multi-channel Bioreactor with non-invasive OD, pH and pO_2 measurement

User instructions

Contents

1. About this edition of the manual

The manual applies to the following versions of personal multi-channel bioreactor:

- RTS-8 plus ..... 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 and 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.

It is the customer's responsibility to validate the sensor and transmitter under end-user conditions according to safety precautions of the application to ensure that the use of the sensor is safe and suitable for the intended purpose.

Biosan is explicitly not liable for direct or indirect losses caused by the application of these measurement systems. In particular it has to be considered that malfunctions can occur due to the naturally limited lifetime of the sensor depending on the respective application. The setup of backup measurement stations is recommended when using the sensors in critical applications to avoid consequential losses. It is the customer's responsibility to install a suitable safety system in the event of sensor failure.

3. General Information

RTS-8 plus 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, fluorescence and luminescence measurement systems, it is possible to register cell growth kinetics, pH and O_2 non-invasively in real time. For pH and O_2, innovative single-use sensor spots are used inside the tubes.

Although O_2 supply is one of the major issues in the cultivation of aerobic organisms, especially in oxygen limited conditions, adequate methods for real monitoring of dissolved oxygen were missing, and sufficient O_2 supply was usually assumed. Innovative non-invasive oxygen sensors integrated in falcon tubes now enable online oxygen monitoring and give new insights into metabolic activities.

The pH is one of the major issues in the cultivation of cells, yeast or bacteria. Cultivation vessels which are sensor limited are widely applied in academic and industrial bioprocess development. As adequate methods for real monitoring of pH were not available, cumbersome at-line sampling was used lacking high data density and interfering with growth. Non-invasive real time pH measurement provides new insights into metabolic activity and changes in metabolic pathways.

Advantages of the sensor spots:

- They are small.
- Their signal does not depend on the flow rate of the sample.
- They can be physically divided from the measuring system which allows a non-invasive measurement.
- They can be used in disposables.

Therefore, they are ideally suited for the examination of small sample volumes, for highly parallelized measurements in disposables, and for biotechnological applications.

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
- Non-invasive O_2 and pH measurement allows for accurate monitoring of metabolic activities

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

- Real-Time cell growth logging
- Real-Time pH and O_2 measurement and logging
- 3D graphical representation of OD, pH, O_2 and growth rate over time over unit
- Pause option
- Save/Load option
- Report option: PDF and Excel
- Connect up to 12 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 plus, Multi-channel bioreactor ..... 1 pce
- Blackout lids with ventilation holes ..... 8 pcs
- TPP TubeSpin® Bioreactor vessels, 50ml ..... 20 pcs
- Sterile TPP TubeSpin® Bioreactor vessels, 50ml, with pH and O_2 sensors ..... 10 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

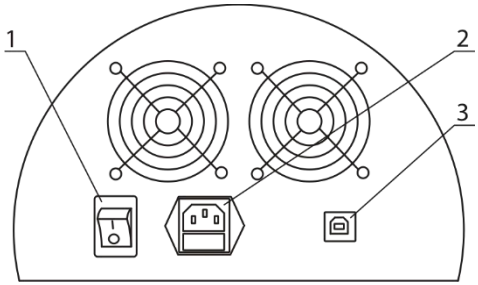

Figure 1. Rear panel of the unit

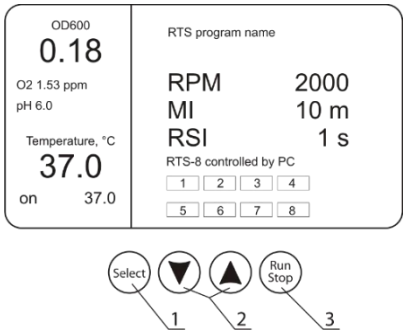

Figure 2. Control panel

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 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 ±0.2 OD_{600}.

4.7 The pH and O_2 sensors with falcon tubes come in a light tight package. It is required to store the tubes in the light tight package and use the tubes only before the initiation of the experiment or calibration.

5. OD optical system 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 PC, 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 (±0.1 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 OD_{600} then the recommended samples are 0 (ddH_2O water or broth media) 1, 2, 3, 4, 5, 6 OD_{600}.

Measure OD at desired wavelength of each cell suspension using a spectrophotometer with proper prior dilutions. The proportionality between OD_{600} and cell density exists only for $OD_{600} \leq 0.4$ (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 plus 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. pH and O_2 optical system and sensors information and calibration

6.1 General information

6.1.1 Optical oxygen sensor.

The light from an LED excites the optical oxygen sensor to emit fluorescence. If the sensor encounters an oxygen molecule, the excess energy is transferred to this molecule in a non-radiative transfer, decreasing or quenching the fluorescence signal. The degree of quenching correlates to the oxygen partial pressure of the analyte in the matrix, which is in dynamic equilibrium with the oxygen in the sample. The decay time measurement is internally referenced

6.1.2 Optical pH sensor.

Optical pH sensors use Dual Lifetime Referenced (DLR) method, which enable internally referenced measurements. A combination of different fluorescent dyes detects intensity changes in the time domain. The luminescence lifetime measured is a superposition of the signals of an analyte sensitive indicator and an inert reference indicator, where both indicators exhibit very different luminescence lifetimes and the luminescence of the analyte sensitive indicator can be supressed by the analyte. It is essential for the pre-calibrated measurements and the easy parallelisation of measurements through the identical calibration of large numbers of sensors.

6.1.3 Temperature dependency of O_2 and pH sensor spots.

It is required to make temperature corrections for the sensor spots at the same working temperature, e.g. 37°C for *E.coli* or 30°C for yeast. For example, at pH 7 a deviation of 0.1 pH per 5°C can occur without added temperature correction.

6.1.4 Limitations.

Caution! Sensors do not stand organic solvents.

The measurements can be influenced by fluorescent molecules like fluorescein or rhodamine.

The pH sensor works best in solutions with ionic strength > 50 mM and buffer capacity > 2 mM. In case of lower salt concentrations or buffer capacity pH may fluctuate or get displayed incorrectly.

Coloured buffers often used for pH electrodes can interfere with chemical optical sensors. Please do not use coloured buffers for calibrating chemical optical pH sensors.

Please note, the pH sensors are not suited for measurements in tap or fresh water.

The sensors need to be equilibrated before usage. In order to do so you have to fill the vessel with your media and wait for at least 60 minutes so that the sensor can equilibrate.

Typical sensor bleaching rate is 0.035 pH per 1000 measurements.

Typical drift of O_2 sensor < 0.03 % O_2 within 30 days (sampling interval of 1 min.).

6.2 **Calibration verification.** Each lot of sensor spots is pre-calibrated, but it is required to make one-point or multiple-point calibration for new sensor spots to increase accuracy or make correction because of 1) ionic strength, 2) temperature, 3) drift, 4) photo bleaching, 5) cross sensitivity. To verify the calibration or to make corrections, please follow the subsequent procedures:

- Connect the device to the computer, launch the software;
- Take a TPP TubeSpin® Bioreactor 50ml tube with sensor spots;
- Add 10 ± 0.1 ml of broth medium with known pH (to obtain sample with known pH, please refer to software manual calibration protocols, pages **32, 36, 40**) and known O_2 (to obtain sample with known O_2, please refer to software manual calibration protocols, pages **45, 46, 48**);
- Close the cap of the tube thoroughly;
- Align sensor spots with socket indicator lines (figure 3);
- Insert the tube into the socket;
- Set the pH and O_2 MI to 1 minute;
- Press the **Play** button in the software;
- The device will start measuring in 1 minute and should complete after 5–60 seconds and pH and O_2 values should appear on the display and software;

If pH and O_2 values equal to known values acquired by software calibration protocols, then the sensors are working as intended.

7. 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 and the sensor spots must align with the two markings (figure 3); this position enables the light from the laser to be transmitted without disruption by different marks presented on the tubes outer surface and it allows for pH and O_2 optics to be on the same axis as the sensor spot.

Figure 3.
Tube positioning

7.1 Connect power cable to electric circuit.

7.2 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.

7.3 Insert the tube into the sockets.

7.4 **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”.

7.5 **Manual mode**.

7.5.1 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 colours 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.
- Purple when pH and O_2 optical module is operating.

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

7.5.3 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).

7.5.4 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.

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

7.7 Disconnect power cable from electric circuit.

8. Recommended methods for microorganism cultivation

8.1 **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)

8.2 **Thermophilic aerobe** *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)

8.3 **Aerotolerant anaerobe** *L. acidophilus:*
0 rpm,
0 s^{-1} RSI,
37° C,
45 ml,
20 min MI

8.4 **Yeasts** *S.Cerevisiae*:
2700 rpm,
1 s^{-1} RSI,
30° C
7.5 ml
20 min., but no less, MI

8.5 **Obligate anaerobe** *B. bifidum*:
0 rpm,
0 s^{-1} RSI,
37° C
50 ml (filled to the max.)
20 min MI

8.6 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.

9. Recommendations for creating personal settings for cultivation of microorganisms. Example results and points to consider

9.1 **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.

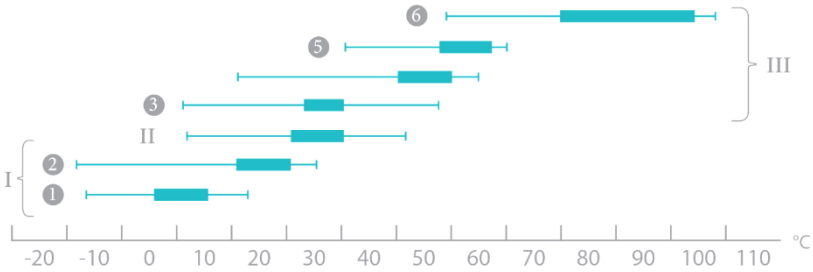

Figure 4. Temperature boarders and optimal growth zones of prokaryotes and their classification.

9.1.1 For psychrophiles, that are cultivated at temperatures of 15°C +-2°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.

9.1.2 For mesophilic microorganisms, the device can be situated at room temperature.

9.1.3 For thermophilic microorganisms, the device can be situated at room temperature.

9.2 **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}.

9.2.1 Legend of experiment (fig. 5.): Multi-channel bioreactor RTS-8 plus 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.

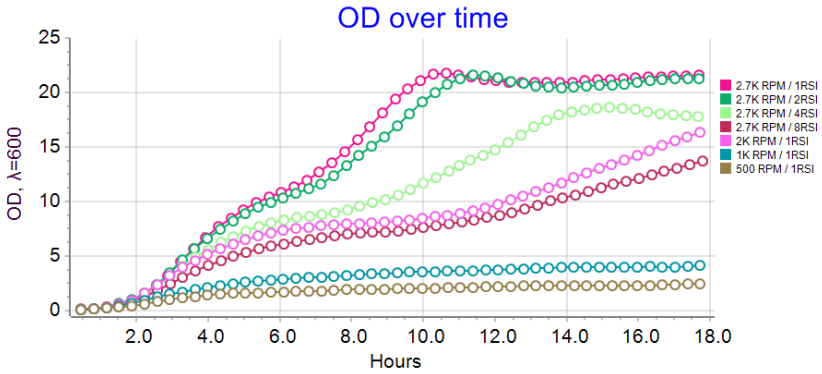

Figure 5. Influence of Interval of Reverse Spinning and RPM on the Growth kinetics ($\Delta OD_{\lambda=600nm}/\Delta t$) vs Time of fermentation (h).

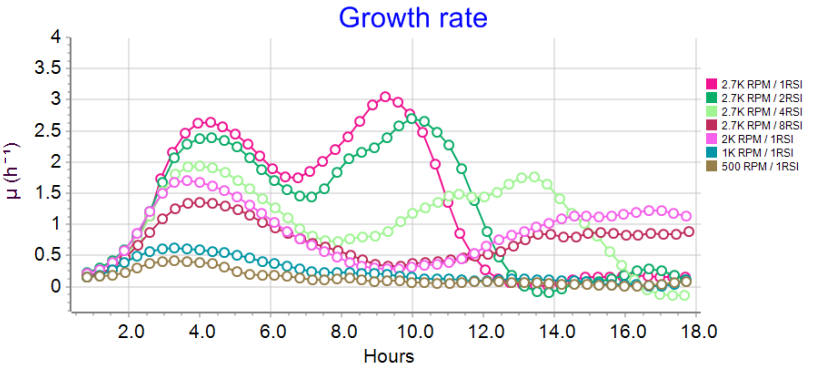

Figure 6. Influence of Interval of Reverse Spinning and RPM on the Growth kinetics ($\Delta OD_{\lambda=600nm}/\Delta t$) vs Time of fermentation (h).

9.3 **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.

9.4 pH and pO_2 measurement example results

The single-use bioreactor falcon tubes were filled with nutrient medium and covered with screw caps provided with special breathing openings, which were closed with a membrane that was semi-permeable to oxygen. Then these 50 ml tubes were placed in RTS-8 plus and the fermentation process was initiated synchronously.

Working volume was 10 ml, the cultivation temperature was 37 ° C, the measurement interval (MI) of the sensors were every 20 minutes, the reverse spin interval (RSI) of the tube 1 time per second, the intensity of rotation of the tubes - according to the signatures to the legends (fig. 9). The aeration intensity was changed by changing the rotation speed or angular velocity (ω) of the bioreactor tube in discrete ranges of ω = 1000 rpm (green curve), ω = 1500 rpm (light green), ω = 2000 rpm (pink curve) and ω = 2700 rpm (purple curve).

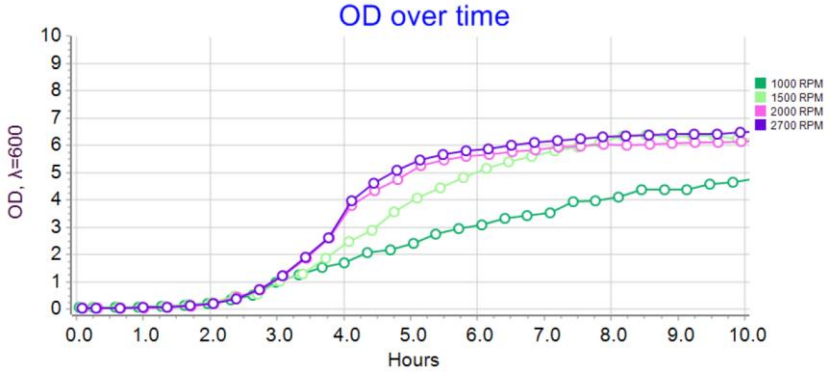

Figure 7. Influence of the rotation speed on the dynamics of cell growth on LB medium

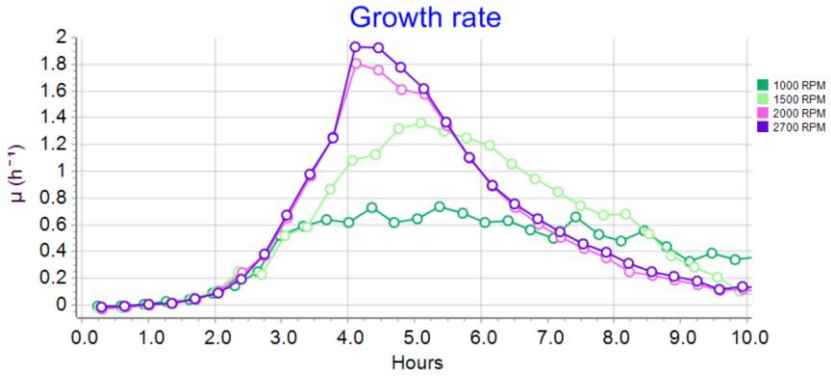

Figure 8. The influence of the speed of rotation of the tube (the intensity of aeration) on the specific growth rate of the biomass of cells.

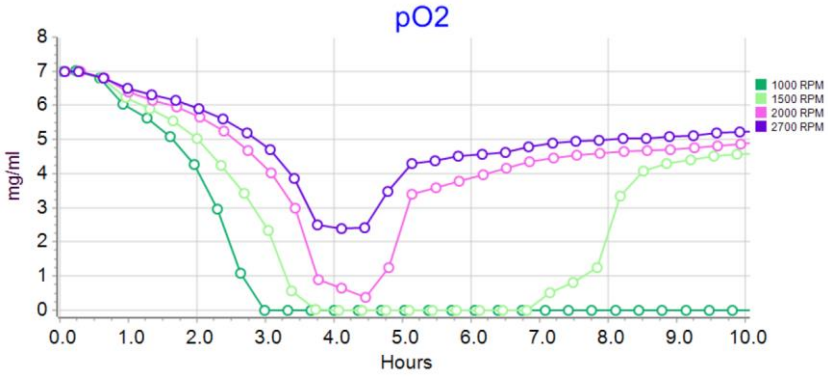

Figure 9. Influence of the speed of rotation of the tube on the dynamics of the change in the concentration of oxygen in the cell suspension.

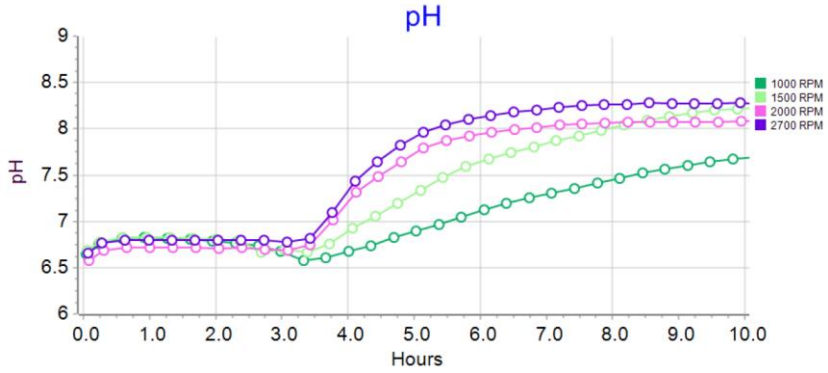

Figure 10. Influence of speed of rotation on the dynamics of pH change in the culture medium

Let us consider the obtained data of the dependence of the change in the rate of growth of oxygen consumption and of pH of the nutrient medium on the rate of rotation of the tube. From the data obtained (see fig. 9), it is clear that an increase in the rotation speed of the tube leads to an acceleration of the growth rate of the optical density of the nutrient medium (OD600) and therefore of cells whose concentration (in mg/ml) corresponds to the values of OD600. The greatest difference in cell concentration is for 4-5 hours of cultivation and then after 10 hours the OD of all variants is compared in the region of 6.0-6.5 OD600, which is the maximum possible yield of biomass of E. coli BL21 cells on the LB medium.

And now let's observe the obtained data in the coordinates of the growth rate of the biomass of cells depending on the time of fermentation (see fig. 10).

From fig. 8 it can be seen that an increase in ω from 1000 to 1500 and then 2000 rpm leads at every step to a 0.7 fold increase in the maximum growth rate of the biomass of cells (the value is expressed in $OD600/h^{-1}$). A further increase from ω 2000 rpm to ω 2700 rpm does not lead to a rise in specific growth rate. Therefore, in a single LB medium at 10 ml of the bioreactor working volume, the aeration conditions achieved at ω 2000–2700 rpm for this strain are not limiting. At the same time, the range below ω 2000 rpm leads to the oxygen limiting conditions. Moreover, the data presented in fig. 8 confirm the above mentioned observations.

From the data presented in fig. 9, it is seen that during the transition of the culture to the logarithmic growth phase, an increase in the intensity of oxygen consumption from the medium consumed for aerobic generation of ATP is observed. If under intensive aeration conditions (corresponding to ω = 2000 rpm and ω = 2700 rpm) the cell culture does not fall into hypoxic shock, then for aeration intensity corresponding to ω from 1500 and below, hypoxia is observed - that is, a state in which the OTR is lower than the intensity of oxygen consumption by the culture. It is interesting to note that this transition is observed at cell concentrations in the medium corresponding to the values given in Table 2.

Table 2 is of practical interest and can serve as an orientation for scale-up of the bioprocess.

Table 2. Dependence of cell concentration at which hypoxia is observed from the intensity of rotation of the tube.

ω (rpm)	μ_{max}	OD600
2700	1.95	4
2000	1.8	3.75
1500	1.35	2.5
1000	0.7	1.75

Now we will consider what happens with the pH of the nutrient medium during fermentation and how this parameter is affected by the intensity of aeration.

It is necessary to note two sections of the pH dependence on the fermentation time: 1) stable pH retention in the initial pH range of 6.8, 2) alkalization of the nutrient medium to pH 8.3 from the moment of oxygenation limitation. From the data obtained, it follows that the change by microorganisms of the pH of the medium is not a response to oxygen limitation (hypoxia) but is the result of another process not associated with aerobic processes. Since the carbon source for the tricarboxylic acid cycle is, as a rule, the ketoacids that result from the deamination and deamidation of the amino acids present in the LB (tryptolytic hydrolysate of the milk protein of casein), then it becomes understandable regarding the alkalization of the nutrient medium to pH 8.3 – the point of equilibrium shift of the ammoniac solution NH_4OH towards ammonia gas NH_3 after 4 to 5 hours of fermentation.

9.5 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).

9.6 **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.

9.7 **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).

10. 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.

10.1 Optical measurement specifications

Light source ..... Laser
Wavelength (λ), nm ..... 850 ± 15
Measurement range, OD_{600} 0–100
Factory calibration measurement range, OD_{600}
- *E. coli* 0–50
- *S. Cerevisiae* 0–75

Achievable user calibration measurement error, OD_{600}
- 0.1–6 ..... ± 0.3
- 6–50 ..... ≤ 5%
- 50–75 ..... ≤ 10%

Real time measurement, measurement interval, min 1–60
Time setting resolution, min 1

10.2 O_2 sensor specifications

Range, % O_2 ..... 0–100%
Accuracy, at 20.9 %, in % O_2 ..... ± 0.4
at 0.2 %, in % O_2 ..... ± 0.05
Drift at 0% O_2 ..... < 0.03 % O_2 within 30 days (sampling interval of 1 min)
Temperature range, °C ..... up to 40
Storage stability[1], months 18
Response time (t90), seconds ..... < 6
Limit of detection, % O_2 ..... 0.03
Resolution, at 20.9 %, in % O_2 ..... ± 0.1
at 0.2 %, in % O_2 ..... ± 0.01

10.3 pH sensor specifications

Range, pH 4.0–8.5
Accuracy at pH 7, pH ..... ± 0.10
Drift, pH per day ..... < 0.005
Temperature range, °C ..... up to 40
Storage stability[1], months 18
Response time[2] (t90), seconds ..... < 120

[1] Provided that the sensor is stored in the dark.

[2] Equilibrated sensor kept in well stirred solution at + 37 °C

10.4 Temperature specifications[1]

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 ..... ± 0.3
Sample temperature accuracy, °C
- 20 °C … 37°C ..... ± 1
- < 20 °C ..... ± 2
- > 37 °C ..... ± 2

10.5 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

11. Maintenance

11.1 If the unit requires maintenance, disconnect the unit from the mains and contact Biosan or your local Biosan representative.

11.2 All maintenance and repair operations must be performed only by qualified and specially trained personnel.

11.3 Standard ethanol (75%) or other cleaning agents recommended for cleaning of laboratory equipment can be used for cleaning and decontamination of the unit.

11.4 Clean the rotor of the device from liquid droplets and possible contamination after finishing fermentation.

11.5 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 11). 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).

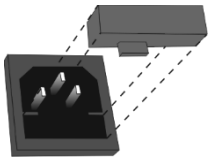

Figure 11.
Fuse replacement

[1] In stable ambient temperature from 20 to 25 °C
[2] Accurate within ±10%

12. Warranty and Claims

12.1 The Manufacturer guarantees the compliance of the unit with the requirements of Specifications, provided the Customer follows the operation, storage and transportation instructions.

12.2 The warranted service life of the unit from the date of its delivery to the Customer is 24 months. For extended warranty, see **12.5**.

12.3 Warranty covers only the units transported in the original package.

12.4 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.

12.5 Extended warranty. For **RTS-8 plus**, the *Smart* 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.

12.6 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

12.7 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 plus, personal multi-channel bioreactor		

13. EU Declaration of Conformity

EU Declaration of Conformity

Unit type	Personal bioreactors
Models	**RTS-1, RTS-1C, RTS-8, RTS-8 plus**
Serial number	14 digits styled XXXXXXYYMMZZZZ, where XXXXXX is model code, YY and MM – year and month of production, ZZZZ – unit number.
Manufacturer	SIA BIOSAN Latvia, LV-1067, Riga, Ratsupites 7 k-2

The objects of the declaration described above is in conformity with the following relevant Union harmonization 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.

I declare that the Declaration of Conformity is issued under sole responsibility of the manufacturer and belongs to the above-mentioned objects of the declaration.

Svetlana Bankovska
Managing director

Signature

07.02.2020.

Date

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