Quick Start Guide

BIOTHRUST

Basic Information
ComfyCell

Probes and sensors

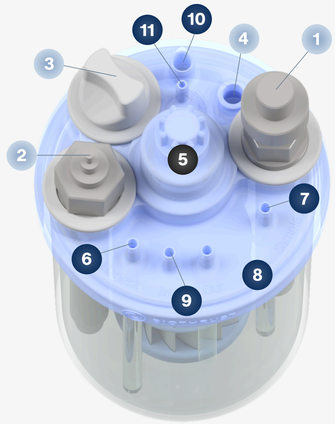
1. PG13.5 Port 1
   Intended for DO probe
2. PG13.5 Port 2
   Intended for pH probe
3. PG13.5 Port 3
   For flexible use
4. Temperature sensor

Stirring

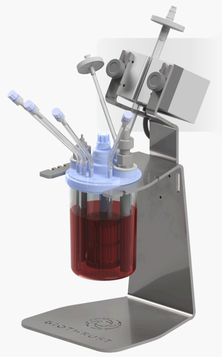
5. Motor

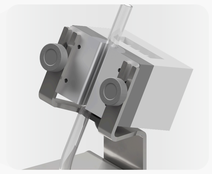
Ports

6. Harvesting
   Collection of content
7. Sampling
   Collection of samples
8. OUT
   Collection of media
9. IN
   Addition of fresh media
10. Exhaust to Condenser
    Pressure release
11. Gas IN
    Entry of air, O_2, CO_2, N_2



Mounting on the stand





1. Place the vessel on the stand using the **side entry** in the vessel
2. Connect the exhaust tube to the condenser

Filters

Diameter	*Pore size*
Gas IN: 25 mm	**0.2 µm**
Exhaust: 37 mm	

Tube sizes and specifications

Tube sizes	*Materials*
Gas IN: 3.0 mm	**Gas IN: silicone**
Exhaust: 7.0 mm	**Exhaust: silicone**
All other: 3.2 mm	**All other: C-flex**

Recommended working volume

150 mL
For optimal scale up

Not recommended

300 mL
For optimal scale up

Recommended agitation

0 rpm — 50 rpm — 100 rpm — 150 rpm — 200 rpm — 250 rpm

Sheer-senstive cells
- iPSC spheroids
- NK cells
- T cells
- Organoids
- iPSC-carriers
- MSC-carriers

Moderately sheer-tolerant cells
- Organoids
- HEK-293 cells
- MSC-carriers

Highly sheer-resistant cells
- CHO cells
- Insect cells
- Microorganisms

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Need some guidance? Contact us at **bioprocess@biothrust.de**

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Calibration & Preparation
ComfyCell

Preparation

Day before

⚠ *Calibrate pH probe beforehand (2-point calibration with pH 4 and pH 7 or 10).*

1. **Pre-wetting**
   - ☐ Fill with DI Water (total vessel volume) and stir for 20 min
     - ⚠ Minimum gas input (for pre-wetting): 100 mL/min
     - ☐ Check for leakage and gas bubbles
   - ☐ After wetting, **discard the water**
2. **Attach autoclavable probes (Dissolved Oxygen (DO), pH, other)**
   - ☐ Vertical Integration: Position probes vertically
   - ☐ Screwing: Ensure probes/BioThrust plugs are securely screwed in
   - ☐ Caps: Ensure caps are placed on electronic connections
3. **Autoclaving** Settings: 121 °C for 20 minutes (~15 PSI) ⚠ *134 °C not suitable*
   - ☐ Clamps: Keep clamps open during autoclaving, close afterwards
   - ☐ Cover filters (use aluminum foil; do not cover the drive shaft)
   - ☐ Put the vessel inside the sterilization bag
   - ☐ Orientation: Autoclave the vessel standing up, take care that tubings are not bent
   - ☐ Remove the vessel from the sterilization bag inside a biosafety cabinet and check if tubes and ports are intact and closed tightly
4. **Connect peripherals**
   - ☐ Place the vessel on the stand using the guideway (check “Mounting on the stand” section)
   - ☐ Gassing: Connect all four gas lines to the filter tube at the fluid wave coupling inlet
   - ☐ Connect the DO probe to the cable and polarize it for **at least 2 h before** starting the calibration
   - ☐ Connect the temperature sensor and the pH sensor
   - ☐ Connect exhaust filter tube to the peltier element by pushing it in
   - ☐ Heating blankets: Attach heating blanket on the vessel
   - ☐ Temperature Sensor: Add 1 mL DI water before inserting the temperature sensor in the tube
     ⚠ *Do not push too hard*

Inoculation

⚠ Check before inoculation

- ☐ **Probe orientation**
  Confirm that probes are not in contact with the stirrer/wall
- ☐ **Probe tightness**
  Ensure all probes are tight under a biosafety cabinet. If needed, retighten
- ☐ **Probe distance**
  Ensure there is no contact between the probes and the wall/stirrer
- ☐ **Cooling**
  Keep exhaust filter vertical (not bent down)

Inoculation start

1. Cooling: Wait for the system to cool down before adding media
2. Add media to the system, use at least as much to cover all probes
3. Start temperature control using 37 °C, the stirrer using 100 rpm and the AIR gassing using 200 cc/min. Wait 20 min, when graph stabilized at maximum go to step 4
4. Calibrate DO
5. Save a cultivation protocol
6. Suggestion: Let the system run until all parameters are stable (~ 1 h)
7. Inoculate once all conditions are stable
   ⚠ Minimum gas input (for inoculation): **10 mL/min**

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