



BIOTHRUST

ComfyCell Incu 300 mL Technical Manual





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Intended Use and Restrictions

This equipment is specifically designed solely for the purposes of research and development and is only made available on a business-to-business basis to the recipient. It is intended as a custom-built evaluation kit designed for professionals to be used solely at research and development facilities for such purposes and only for temporary use in these laboratories.

BioThrust GmbH

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Safety



If BioThrust equipment is used in a manner not specified by BioThrust, the protection provided by the equipment may be compromised.



The power cord is the main electrical disconnect for the ComfyCell Incu. To remove power from the unit, unplug the power cord. Do not position the unit in a way that it is difficult to unplug the power cord even when inside an incubator.



To avoid damage or injury, only use power supplies and accessories provided by BioThrust. Only use vessels manufactured by BioThrust to guarantee compatibility and performance.



Do not use the equipment in hazardous atmospheres or with hazardous materials for which the equipment is not designed.



If biohazardous materials are used with this device, follow all applicable local, state/provincial, and/or national regulations, including identification of samples with the biohazard symbol. Wear appropriate protective gear.



If the equipment has been used in a biohazardous environment, it must be decontaminated according to all applicable local, state/provincial, and/or national regulations prior to shipment or disposal.



About the BioThrust ComfyCell Incu

The BioThrust ComfyCell Incu (“Incu”) bioreactor is a Single-Use 3D cell culture device, intended primarily for culture of cells in cell & gene therapy applications such as PSCs, MSCs or T-Cells. The system consists of a 300 mL Single-Use Vessel with the proprietary BioThrust Membrane Stirrer (“MemStir”) and a non-disposable Base Unit that holds the vessel. The 300 mL reactor is designed for R&D or small-scale process development projects and is suitable for research use only.

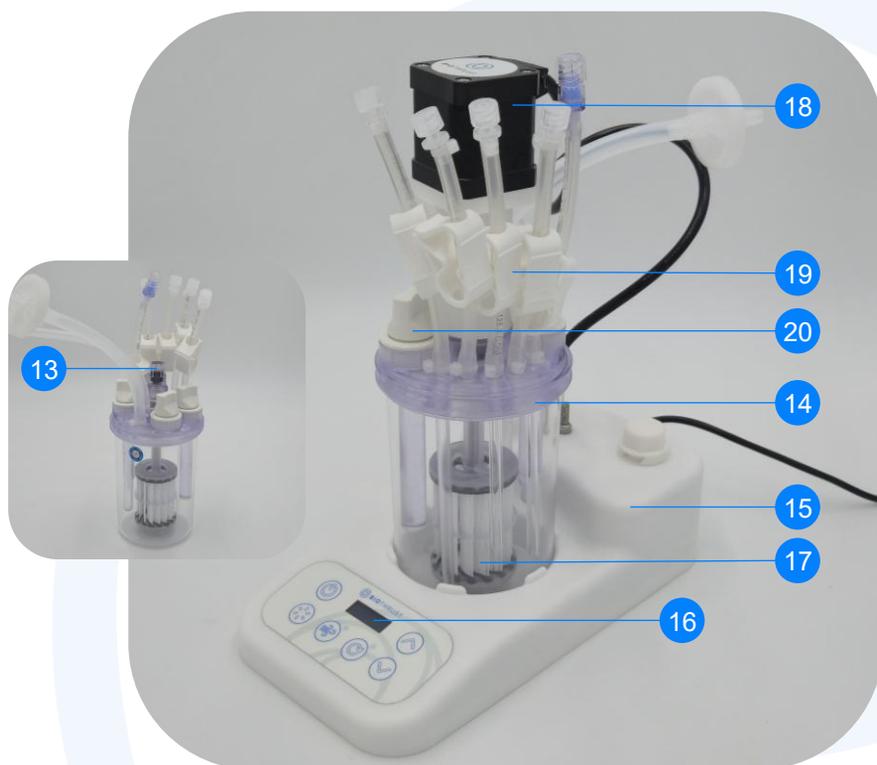
The Incu Base Unit is comprised of an injection-molded plastic housing which contains electronics and an aeration pump. The Base Unit’s plastic housing is sealed for use in up to 100% humidity conditions and is constructed to be cleanable with 70% v/v isopropyl alcohol, or equivalent. The Base Unit may be powered using the included regional power adapter at 100-240 VAC; 50/60 Hz.

The Single-Use Vessels, containing the MemStir, are constructed from 3D printed plastic (headplate) and injection-molded plastic (vessel body) and medical adhesives. The Membrane Stirrer’s hollow fibers are made from PMP. The entire Vessel is to be sterilized by a single autoclaving cycle, either by BioThrust or on-site by the customer. All product-contact surfaces in the Single-Use Vessels consist of components certified to be medical grade or inherently safe for biological contact. The system itself is therefore suitable to prepare for clinical processes but not to be used outside of R&D settings.

The agitation rate of the MemStir in the Single-Use Vessel is controlled by the speed adjust buttons on the Base Unit and is displayed on the digital display in the center of the device. Active aeration and vessel illumination can be toggled through their respective buttons on the Base Unit with LEDs next to the buttons indicating activation. To regulate temperature, pH, and dissolved oxygen (DO) levels in the culture, the system must be placed in an incubator with appropriate temperature and gas controls.



ComfyCell Incu Features





- 1 External Power Cord Input**
Supplies power to the Base Unit from an A/C power source. Suitable for 100-240 VAC.
- 2 Gas OUT for Vessel**
Tube to let spent gas exit the Vessel. Ensure it is not bent and unobstructed during operation.
- 3 Gas IN for Vessel**
Tube to connect the Base Unit's pump with the Single-Use Vessel's Gas IN. Connect tubing well before usage to ensure active aeration.
- 4 Gas Intake for Base Unit**
Provides gas to the pump and therefore MemStir. Always keep unobstructed.
- 5 Motor Resting Socket**
Socket that accommodates the motor while unused for easier handling.
- 6 Button: Power On/Off**
Turns the whole system on (single press) or off (hold longer press until confirmed on display).
- 7 Button: LED Lights On/Off**
Turns the LED light on or off with a single press. LEDs automatically turn off after a certain time.
- 8 Button: Aeration Pump On/Off**
Turns the pump supplying the incubators atmosphere to the MemStir on/off by single press.
- 9 Button: Enable RPM Change**
Press once to allow RPM changes and enable the RPM change buttons, indicated by a flashing RPM display. Hold to set RPM to 0.
- 10 Button: Decrease RPM**
Single press or hold to decrease RPM down to -150 RPM (full speed counter-clockwise agitation).
- 11 Button: Increase RPM**
Single press or hold to increase RPM up to 150 RPM (full speed clockwise agitation).
- 12 LED Lights**
LED plate providing enhanced visualization of cell cultures inside the Single-Use Vessel. Ideal for taking pictures.
- 13 Motor Coupling**
Connects to the motor to drive the Membrane Stirrer in the Single-Use Vessel.
- 14 Single-Use Vessel**
Contains and mixes up to 300 mL (min. 150 mL) of cell culture at a controlled speed.
- 15 Bioreactor Base Unit**
Provides power to the motor, aeration pump and can illuminate the Single-Use Vessel via LEDs.
- 16 Digital Display**
Indicates the Membrane Stirrers RPM and provides input feedback when using other features.
- 17 Membrane Stirrer**
Our proprietary MemStir, which combines media aeration and agitation into one sleek component.
- 18 Motor**
Top-loaded motor that spins the Membrane Stirrer clockwise or counter-clockwise at speed between 0 RPM and +/-150 RPM.
- 19 Feeding Tube with Clamp**
4 x Luer ports for media change & sampling. Available at various depths. Autoclave according to instructions!
- 20 PG 13,5 Port**
3 x PG13,5 ports with dummy probes. Dummy probes are essential for consistent fluid dynamics during scale up!



Technical Specifications of ComfyCell Incu

Base Unit	
Size: <ul style="list-style-type: none"> Width Depth Height Weight (with motor) 	<ul style="list-style-type: none"> 12.5 cm (4.9 inch) 23.2 cm (9.1 inch) 8.5 cm (3.3 inch) 0.87 kg (1.92 lb)
MemStir Agitation: <ul style="list-style-type: none"> Mechanism RPM Range Display 	<ul style="list-style-type: none"> Top-loaded mechanical drive -150 to +150 RPM OLED
Operational Ranges: <ul style="list-style-type: none"> Temperature Humidity Environment 	<ul style="list-style-type: none"> Up to 40 °C 5-100% Indoor usage
Construction and Materials	Injection-molded plastic shell, electrical components
Illumination	15 LEDs 7W (6000K)
Aeration	High frequency pump
Single-Use Vessel with MemStir	
Size: <ul style="list-style-type: none"> Diameter Height (on base & motor) Weight 	<ul style="list-style-type: none"> 8.5 cm (3.3 inch) 27.1 cm (10.7 inch) 0.4 kg (0.88 lb)
Working Volume	150 mL or 300 mL
Number of Ports <ul style="list-style-type: none"> Luer PG13,5 	<ul style="list-style-type: none"> 4 (Sampling & Harvesting) 3 (Optional, contain dummy probes)
Liquid Transfer and Sampling	Manual via vessel ports
Materials	PMP, PA12 vapor smoothed, stainless steel, medical grade adhesives



Unpacking & Assembling

- A. Confirm that all components of your ComfyCell Incu package have been delivered in good condition, in the requested quantity, and are of the correct MemStir type for your process (Aggregates or Single-Cells). Verify with BioThrust if you are unsure!



Single-Use Vessel(s)



Base Unit



Motor & Cable

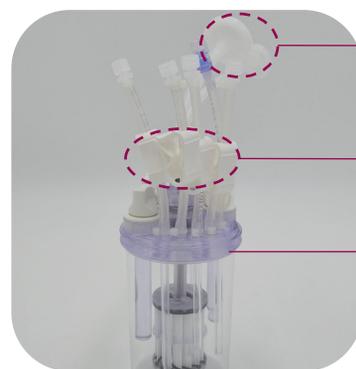
- B. Wipe down the Incu Base Unit with 70% ethanol before placing it in a sterile environment such as an incubator!
- C. Place the Incu Base Unit in an incubator and route the extension cable to a convenient location. Ensure at least 28cm of height in the incubator to accommodate the Base Unit, vessel & motor! You may need to adjust separator panels.
- D. Make the connection between the adapter extension cable and power supply exiting the ComfyCell Incu outside of the incubator. Plug the power supply into an electrical outlet.
- D. Place a ComfyCell Single-Use Vessel on the Base Unit (after sterilization if in a sterile environment) and connect the motor with the vessel's coupling by placing it on top. Ensure that the motor slides completely down so that its teeth interlock properly.
- E. Connect the vessel's aeration tubing (Coming from "Gas IN" at the vessel) with the gas exit on the Base Unit and ensure it is fully screwed on.





Sterilizing

- A. Add DI Water (300 mL) using one of the designated “Media In” probes or add directly via one of the headplate’s PG 13,5 ports using a serological pipette.
- B. Place the vessel in the circular recess of the Base Unit, connect the motor and Gas IN tube and ensure proper fit.
- C. Press the power button to turn on the Incu.
- D. Press the RPM change button to enable RPM adjustments (display is flashing). Select +60 RPM with the RPM adjust buttons and confirm by pressing RPM change again. The display will stop flashing and the MemStir will spin up.
- E. Press the pump button to enable the Base Unit’s pump and start active aeration of the vessel. You should hear a high-pitched noise and see the LED next to the button glow.
- F. Run for 20 minutes to pre-wet the membrane and check for any gas leaks. If bubbling comes from the MemStir, a membrane may be damaged as aeration normally occurs fully diffusively!
- G. Remove the water from the vessel via a harvesting probe (for example with the help of a 50 mL syringe).
- H. Cover both air filters (Gas IN & Gas OUT) with aluminum foil. You do not need to cover the drive shaft or the ports.
- I. Open all clamps.
- J. Put the vessel in the sterilization bag. Take care that tubings are not bent during autoclaving.
- K. Autoclave the vessel standing upright for 20 min at 121° C (~15 PSI). Do not exceed this temperature! 134° C will damage the vessel! The end temperature should be 70° C.
- L. Quickly remove the vessel from the autoclave and, inside a clean bench, take it out of the sterilization bag to verify all tubes are still connected. Next, remove the aluminum foil covering the air filters.
- M. Let the vessel cool down and then close the tube clamps.



! Cover both air filters with aluminum foil

! Open all clamps before autoclaving

! Only autoclave vessel standing upright at 121° C (~15 PSI) for 20 minutes



Preparing & Inoculating

Preparing

- A. Fill 250 mL of freshly prepared media into the vessel intended for cell culture using one of the feeding probes or add directly using one of the headplate's PG 13,5 ports using a serological pipette.
- B. Place the vessel in the circular recess of the Base Unit, connect the motor and AIR IN tube and ensure proper fit.
- C. Place the sterile Incu system in the incubator
- D. Set RPM to +60 and let the Incu run for 60 minutes to stabilize your media to the incubator temperature as well as to the atmospheric conditions inside the incubator.

Inoculating

- A. During the stabilization step to condition the media in the Incu, collect your cells and transfer them into 50 mL of your freshly prepared media intended for your expansion run.
- B. When the stabilization is completed and your cells are ready to be transferred, reduce RPM to 0 or turn off the Base Unit entirely.
- C. Remove the vessel from the Incu Base Unit and place it in a clean bench. Keep the Base Unit in the incubator.
- D. Transfer your cells into the vessel using the feeding tubes (with a syringe for example, or alternatively through the PG 13,5 port on the headplate using a serological pipette).
- E. Close all the tubes/ports and place the vessel back on the Incu Base Unit inside the incubator.
- F. Re-connect motor and air supply.



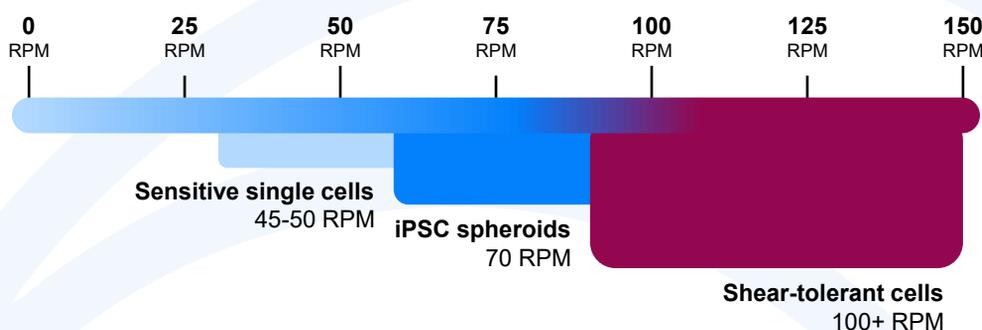


Operating

This chapter assumes you have followed the instructions on how to sterilize, prepare and inoculate the Incu and now have a switched off but otherwise prepared system standing in your incubator.

- A. Press the power button to turn on the Incu.
- B. Press the RPM change button to enable RPM adjustments (display is flashing). Select the RPM suitable for your process – the MemStir will spin up.

Suggested RPM setpoints



 **Questions?** Contact BioThrust to discuss your unique process set up!

- C. Press the pump button to enable the Base Unit's pump and start active aeration of the vessel. You should hear a high-pitched noise and an LED indicating the pump is running.
- D. To visualize flow patterns or cells inside the vessel you can turn on the LED lights with the LED toggle button. Avoid or limit LED usage when working with light-sensitive processes. The LEDs will switch off automatically after a short while.
- E. To adjust speed while the MemStir is already spinning, press the main RPM button until the display flashes, then use the RPM change buttons to proceed as before by adjusting RPM. Press the main RPM button again to confirm. The MemStir will gradually adjust to your new setting to avoid disruption.
- F. Turning off the system is done by holding the power button until the display signals that the device is shutting down. It is not necessary to stop the Stirrer before turning off the device. To turn off the stirrer separately, hold the motor button.



Sampling

The MemStir does not need to be stopped during sampling & harvesting and the Incu can remain in the incubator. This allows you to maintain accurate culture conditions.

- A. To remove a sample from the Single-Use Vessel, use a syringe and choose a Luer Port. You can use any of the ports for “Harvesting”, “Sampling” or “Media Out”. Your choice depends on what depth of the bioreactor you want to take a sample from.
- B. First, disinfect the safe flow using 70% ethanol or similar.
- C. Connect the syringe and draw the necessary amount of sample and disconnect the syringe.
- D. Rinse the safe flow again with 70% ethanol and close the lid.





Media Exchange

- A. For media exchange, choose a Luer port depending on the length/height of the internal tubing needed (e.g., for partial or half media exchanges). Do not use the “Gas In” or “Gas Out” ports for media exchange.
- B. We recommend stopping agitation and performing the media changing procedure inside a biosafety cabinet to minimize the risk of contamination.
- C. Using a 50 mL Luer-lock syringe, connect to the Luer port of the selected harvesting tube and aspirate the cell-containing medium. Transfer the collected volume into one or more collection tubes (50 mL or larger, as required).
- D. Repeat the procedure until the desired culture volume has been removed. When using the Harvest port (intended for full media exchange), tilting the vessel will maximize volume recovery.
- E. Centrifuge the harvested sample. During centrifugation, fresh medium can already be added back to the bioreactor to prevent cell drying. For a total culture volume of 300 mL, we recommend adding 250 mL of fresh medium.
- F. After centrifugation, discard the spent medium and resuspend the cell pellet in fresh medium (e.g., 50 mL for the volumes recommended above).
- G. Transfer the resuspended cells back into the bioreactor using one of the available Luer ports.
- H. Finally, place the vessel back onto the Incu base unit and reconnect the motor and gas-in tubing to continue the expansion run.





Harvesting

- A. To harvest the culture medium, use the Luer port designated “Harvest”, as this enables recovery of nearly 100% of the culture volume.
- B. We recommend stopping agitation and performing the harvest inside a biosafety cabinet to minimize the risk of contamination.
- C. Using a 50 mL Luer-lock syringe, connect to the Luer port of the harvesting tube and aspirate the cell-containing medium. Transfer the collected sample into one or more collection tubes (50 mL or larger, as required).
- D. Repeat the procedure until the entire culture volume has been collected. Slightly tilting the vessel can help maximize volume recovery.
- E. Centrifuge the harvested sample and proceed with downstream experiments or analyses.





System Handling & Vessel Storage

All parts of the ComfyCell Incu are maintenance-free. There are no periodic replacement parts. Please contact BioThrust if you experience errors or suspect damage.

Base Unit

- Can be cleaned with 70% ethanol, no other maintenance is required.
- Do not attempt to open the Base Unit under any circumstance as this leads to a loss in insulation!
- If not in use, remove Base Unit from the incubator and unplug it completely to extend its service life.

Single-Use Vessel 300 mL

- Must be stored in a dry and dark environment to avoid radiation / UV-light based damage to the membrane!
- Shelf life of vessels is several months under correct conditions.
- Vessels can only be autoclaved once. You may attempt to reuse them, especially if using sterilization methods like ethanol cleaning or simple washing – however, any reusing voids BioThrust guarantee and is done at own risk.



Frequently Asked Questions

ComfyCell Incu not powering up?

- Verify that the ComfyCell Incu is plugged into a functional electrical outlet. If in doubt, try multiple outlets.
- Verify that any intermediate power strips or connectors are also switched on.
- Verify that the extension cable is connected securely.

Membrane Stirrer not spinning as intended?

- Verify that the motor has slid all the way down on the coupling and the gearing is properly interlocked. Rotate the motor on the axis to confirm that it's all the way down.

Pump not providing air?

Note: Aeration occurs at a molecular level without visible bubbles!

- The pump will emit a high-frequency sound when active. Ensure the Tubing between the Base Units Gas Exit and Single-Use Vessels Gas In port is tightly connected. Before inoculation verify the membrane is undamaged.

LED lights not staying on?

- To avoid accidental overheating or damaging light exposure, the LEDs are programmed to turn off automatically after a few seconds.

Device produces humming noise?

- A consistent, high-pitched humming sound typically indicates that the pump is activated. Confirm by checking if the LED next to the air pump toggle is on.

MemStir wobbling slightly?

- Since our Membrane Stirrers are assembled by hand, slight manufacturing variations may occur, potentially causing minor wobbling at higher RPMs. This is not a cause for concern and will not harm cells in any way or alter process conditions.



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