

Standard Operating Procedure

CellMembra - ver 1

The following is recommendations, guide lines only. Originate from December 2015. We suggest you develop your own Standard-Operation-Procedure (SOP).

Purpose

Perfusion cultivation with mammalian cell line in a Single-Use-Bioreactor (SUB) for expressing a product. Cell retention performed by a single-use Cross-Flow-Filter (CFF). Broth circulation performed by an integrated Single-Use-Pump (SUP). Operating after the Pulsating-Tangential-Flow (PTF) principle.

Benefits

Volume and velocity fully selectable from the Lachesis units. Data show are measured and 100% accurate. No show data is estimated!

System setup

CellMembra family (500 ml and 2.1 and 3.2 VV) members all require the Lachesis controller (not part of the SUB kit) from www.perfusecell.com. Lachesis is supplied with a re-usable triangular laser instrument (red little box), which senses online the position of the piston in the Air Column SUP with better than 0.5 mm accuracy. CellMembra further include:

- Agitation performed by a range of mechanical devices controlled from a Process-Control-System (PCS not part of the kit)
 - Temperature control to be used combined with a PCS
 - pH control to be used combined with a PCS
 - DO control to be used combined with a PCS
 - Media exchange performed by one or more pumps and a PCS
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CellMembra P-SUB package include:

- Impeller, hoses, connections, aeration, possible condenser / foam collection bottle
 - CFF
 - SUP – Single-Use-Pump
 - pH sensor from either Hamilton or Broadley-James
 - VisiWell for the VisiFerm DO sensor
 - Potentially two liquid level sensors (plus one ground)
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Preparation of extra traditional **Re-Usable-Sensors (RUS)** to be installed under sterile conditions:

- Glucose, Lactate, pCO₂ – PG 13,5x120-225 sensors, pre-sterilised (not part of the kit).
- Biomass PG 13,5x120-225 sensor and pre-amplifier (not part of the CellMembra kit) on the biomass sensor socket and cabling to the appropriate instrumentation (not part of the kit).

Preparation of **Single-Use-Sensors (SUS)** in CellMembra, which is: pH, dO₂, biomass, other sensors:

- Insure you have the appropriate cabling to the pre-installed classical pH SUS. Calibrate appropriate the sensor according to the calibration recommendation from the manufacturer.

- Install the Hamilton classical dO₂ VisiFerm sensor body (not part of the CellMembra kit) without the; 1. Cap, 2.the tiny O-ring. 3. the large O-ring under the PG13.5 threaded nut into the well. Insure you have the appropriate cabling between the VisiFerm and your PCS. Install and calibrate appropriate the sensor according to the calibration chart from Hamilton.

Pre-cautions:

- DO NOT heat up the SUB until after media is added!
- Insure stable location of the SUB
- DO NOT dry-run the integrated impeller for more than few minutes at less than 200 RPM
- The product is designed for single-use only and will be damaged during treatment with pure ethanol or temperature above 50°C.

Prep before start-up

1. Install the CellMembra accurately in any of: the *DASbox*[®], bioBlock, on CerCell Magnetic-Stirrer-Table (MST), in CerCell Servo-Motor-Support (SMS), on laboratory stirrer table or the like
2. Insert into VisiWell and calibrate the Hamilton VisiFerm dO₂ sensor with air in the reservoir
3. Connect appropriate sized fresh media supply to port #? and used media bag to port #?
4. Add 5-10 ml water or glycerine oil into the non-invasive temp sensor port #?
5. Mount the thermocouple in the non-invasive port #?
6. Mount appropriate heating blanket around the vessel
7. Pump media to the SUB. Insure the sensors are fully covered with media. Remember the inoculation volume will add to the SUB level!
8. Test pumps for adding fresh media and removing broth
9. Connect sparging gas supply to port #? sterile filter, install optionally filter heater and secure the filter orientation vertically
10. Connect optionally head space flushing gas supply to port #? sterile filter and secure the filter mechanically vertical
11. Insure appropriate support for exhaust gas filter and optionally exhaust gas condenser
12. Initiate the agitation and increase the rpm until 100 RPM
13. Allow heating of the SUB and see constant temperature is obtained
14. Calibrate the pre-installed Single-Use-Sensor's according to the calibration recommendations.
15. Test functionality and program Lachesis
16. Inoculate through port #? and start the cultivation in batch mode
17. Having reached the desired biomass level start up the Lachesis
18. Remove broth according to calculations

Liquid level control

On Process-Control-Systems:

- with balancer input
- with no balancer input

Inoculation of cells

High density – for the new user!!

Insure the cells are suspended carefully at high density in 50-100 ml media. Cell numbers to be inoculated depends heavily on the cell line and range for CHO cells – suggested 1-2x10E+06 cells.

Cell densities of +75 mio/cell/ml should be reached – if this is you target.

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