Standard Operating Procedure CellMembra-mini - ver 1.1

The following is recommendations, guide lines only for the CellMembra-mini 500 ml Vessel Volume P-SUB. Date December 2017. We suggest you develop your own Standard-Operation-Procedure (SOP).

Purpose

Perfusion cultivation with mammalian cell line in a Perfusion-Single-Use-Bioreactor (P-SUB) for expressing a product. Cell retention performed by a single-use Hollow-Fiber-Filter (HFF). Broth circulation performed by the integrated Clio One-way-Single-Use-Pump (O-SUP). The CellMembra-mini P-SUB is designed to operate after the Pulsating-Tangential-Flow (PTF) principle.

Benefits

Volume and velocity fully selectable from the Clotho Drive Units. Data show are measured and 99% accurate. No show data is estimated!

System setup

CellMembra family (500 ml and 2.1 and 3.2 VV) members all require the Clotho Drive Unit (not part of the P-SUB kit) from <u>www.perfusecell.com</u>. Clotho is supplied with a re-usable triangular Laser instrument (red little box), which senses online the position of the diaphragm position inside the Clio O-SUP with better than 1% 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
- Bio-mass control by Aber equipment
- Media exchange performed by one or more external pumps and a PCS

CellMembra P-SUB package include:

- SUB impeller, hoses, connections, aeration, aeration filter, exhaust filter
- HFF Hollow-Fiber-Filter MF type supplied dry
- O-SUP model Clio One-way-Single-Use-Pump
- Port 1 pH sensor from either Hamilton or Broadley-James
- Port 2 VisiWell for the VisiFerm DO sensor
- Port 3 Bio-mass PICO SUS
- Port 4 Liquid level sensor (plus one ground) set to 250 ml height

Preparation of **Single-Use-Sensors (SUS)** in CellMembra, which is: pH, DO, bio-mass, level:

- 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.

• PICO biomass sensor requires the FUTURA amplifier from Aber (not part of the kit)

Thermal control:

The Heating-Support-Foot (HSF) pn 2262 is designed specifically for the CellMembra-mini. HSF fits on the MST or any lab magnetic stirrer table. Connect to relevant thermal input – electricity or water.

Pre-cautions:

- DO NOT heat up the SUB until after media is added!
- Insure stable location of the SUB like in the Heating-Support-Foot
- DO NOT dry-run the integrated impeller for more than few minutes
- The product is designed for single-use only damaged by temperature above 50°C.

Prep before start-up

- 1. Install the CellMembra-mini accurately in in PerfuseCell HSF on PerfuseCell Magnetic-Stirrer-Table (MST)
- 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. Mount the thermocouple in the HSF
- 5. Insure appropriate HSF connection for thermal control
- 6. Pump media to the P-SUB. Insure the sensors are fully covered with media. Remember the inoculation volume will add to the P-SUB level!
- 7. Test external pumps for adding fresh media and removing broth
- 8. Connect sparging gas supply to port #? sterile filter, install optionally filter heater and secure the filter orientation vertically
- 9. Connect optionally head space flushing gas supply to port #? sterile filter and secure the filter mechanically vertical
- 10. Insure appropriate support for exhaust gas filter and optionally exhaust gas condenser
- 11. Initiate the agitation and increase the rpm to 100 RPM with downward media flow
- 12. Allow heating of the P-SUB and see constant temperature is obtained
- 13. Calibrate the pre-installed Single-Use-Sensor's according to the calibration recommendations.
- 14. Test functionality and program of Clotho
- 15. Inoculate through port #? and start the cultivation in batch mode
- 16. Having reached the desired biomass level start up Clotho re-circulating broth
- 17. Remove broth according to calculations

Liquid level control

On Process-Control-Systems:

- with balancer input
- with liquid level input

Inoculation of cells

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 +50 mio/cell/ml should be within you reach – if this is you target. Read the Tips & Trick file with guide lines

END