

2.2 CELLine CL 1000

CELLine
classic



2.2.1 Required Material and Preparation

- CELLine CL 1000 Bioreactor
- Standard 25 ml serological pipettes with Pipetting aid
- Preculture of totally 25×10^6 viable cells
- 350 ml of fresh nutrient medium suitable for your individual cell type and equilibrated to the desired culture temperature (see 3.1).
- 5 ml of fresh complete medium

For more information on media composition please also refer to general note 3.2.

2.2.2 Equilibration of CELLine

Day 1 In order to obtain optimal performance of CELLine put 50ml of nutrient medium into the medium compartment and let the semi-permeable membrane equilibrated for at least 5 minutes (see 3.3).

2.2.3 Preparation of Inoculum

Obtain 25×10^6 viable cells from a pre-culture in log growth phase and suspend the cells in 15 ml fresh medium resulting in a minimal concentration of about 1.5×10^6 viable cells / ml (see 3.4).

2.2.4 Inoculation of CELLine

Loosen the big, front cap of medium compartment in order to prevent air lock. Aspirate the 15 ml cell suspension into a serological pipette, open the cell compartment and inoculate the cell compartment by inserting the pipette into the black silicone cone.

It is important to minimize the introduction of air bubbles into the cell compartment during seeding. In case air gets trapped within the cell compartment try to carefully remove the big bubbles by carefully drawing them back into the pipette together with fluid. Close the cell compartment by completely tighten the cap.

After seeding add 975 ml of equilibrated medium into the medium compartment and then completely tighten both caps. Place the CELLine into a standard CO_2 incubator under culture conditions appropriate for your individual cell type.

2.2.5 Culture monitoring (optional)

- Day 3** After 72 hours, take a sample from the cell compartment for assessment of cell density and viability, expression levels of recombinant protein or determination of other individual critical culture parameters. This is especially important when culturing a new cell type in order to establish a working protocol.

2.2.6 Cell compartment harvest and Spilt back

- Day 7** In general, the first harvest is recommended 7 days after inoculation (see also note 3.5).

In order to harvest the cells, simply pour off and discard all medium from the medium compartment.

Avoid to shake the CELLine during this process (see note 3.6)

Loosen the white medium compartment cap

Gently harvest all liquid from the cell compartment by aspirating content with a 25 ml serological pipette. Slowly pipette the liquid up and down several times to thoroughly mix the cell suspension. The cell compartment will comprise about 15 ml cell suspension with the individual secreted product. Due to osmotic flux of liquid from the medium - to the cell compartment, the total volume might be slightly increased (see note 3.7).

Take 3 ml of mixed cell suspension and add to 12 ml fresh complete medium (1:4 Split Back) and gently return the 15ml of cell suspension back into the cell compartment (see note 5.5)

Remove any air bubbles as described above. Tighten the green medium compartment cap.

Add 1000 ml of fresh, preheated nutrient medium to the medium compartment. Place CELLine back into the incubator until next harvest.

2.2.7 Harvesting Cycles

- from Day 14** Consecutive harvests can approximately be made every 5 to 7 days (depending on the individual application and cell type used, also see note 3.5). All harvests are performed as outlined above and should include a change of the culture media.

Periodically, cells can be monitored for growth and production by removing a small sample from the cell compartment. If the CELLine Bioreactor is handled with care and the sterility barrier is not broken individual cultures can be maintained over several months.