

2 Standard Operating Instructions

2.1 CELLine CL 350



2.1.1 Required Material and Preparation

- CELLine CL 350 Bioreactor
- Standard 10 ml serological pipettes
- Pipetting aid
- Preculture of 8×10^6 viable cells
- 350 ml of fresh nutrient medium suitable for your individual cell type and equilibrated to the desired culture temperature (see 3.1).
- 5ml of fresh complete medium

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

2.1.2 Equilibration of CELLine

Day 1 In order to obtain optimal performance of CELLine put 10 ml of nutrient medium into the medium compartment and let the semi-permeable membrane equilibrate for at least 5 minutes (see 3.3).

2.1.3 Preparation of Inoculum

Obtain 8×10^6 viable cells from a pre-culture in log growth phase and suspend the cells in 5 ml fresh medium resulting in a minimal concentration of 1.5×10^6 viable cells / ml (see 3.4).

2.1.4 Inoculation of CELLine

Loosen the green cap of medium compartment in order to prevent air lock. Aspirate the 5 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 340 ml of equilibrated medium into the medium compartment and then completely tighten both caps. Place the CELLine into a standard CO₂ incubator under culture conditions appropriate for your individual cell type.

2.1.5 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.

2.1.6 Cell compartment harvest and Spilt back

- Day 7** In general, the first harvest is recommended 7 days after inoculation. For more information please refer to general 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 green medium compartment cap

Gently harvest all liquid from the cell compartment by aspirating content with a 10 ml serological pipette. Slowly pipette the liquid up and down several times to thoroughly mix the cell suspension. The cell compartment will comprise about 5 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 1 ml of mixed cell suspension and add to 4 ml fresh complete medium (1:4 Split Back) and gently return the 5 ml of cell suspension back into the cell compartment (see note 3.5).

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

Add 350 ml of fresh, preheated nutrient medium to the medium compartment. Place CELLine back into the incubator until next harvest.

2.1.7 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, see also 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.