

3 General Notes for optimal use

3.1 Media preparation

Warm the medium to 37°C before adding into CELLine. The large volume necessitates warming medium to prevent exposure of cells to reduced temperature for long periods. It also helps to avoid condensation and to eliminate temperature fluctuations inside the incubator.

3.2 Media composition

In general there is no need to specifically adapt the media composition for growing cells in CELLine, but the two compartment configuration provide the user additional flexibility to normal cell culture disposables.

Due to the high cell densities which are achieved in CELLine it is recommended to use high glucose medium with a minimal concentration of 2.5 g/l. The described standard protocol was established using concentrations of 4 to 5 g/l D-glucose and 4 mM L-glutamine.

Serum use in Nutrient medium and Complete medium

The terms nutrient medium and complete medium refer to the fact that when working CELLine the medium used in the cell compartment has not to be the same as in the medium compartment. In the standard protocol, only the cell compartment is supplemented with about 5 to 15% FCS, whereas the nutrient medium contains only 1% FCS or is entirely serum free.

Serum free media

For some applications it is necessary to avoid the addition of FCS to the cell compartment and users report excellent results with synthetic medium in CELLine. Because most synthetic, serum free medium formulations contain growth stimulatory components of small molecular weight, which may cross the semi-permeable membrane (10 kDa cut-off), it is recommended to use serum free medium in both the cell compartment and the medium reservoir.

An alternative strategy is to supplement the nutrient medium with serum while using serum free medium in the cell compartment. Thereby the cells would be still protected from potential contaminants in the serum by the 10 kDa sterility barrier provided by the semi-permeable membrane.

3.3 Membrane equilibration

It is important to wet the semi permeable membrane prior to inoculation to assure that the membrane is compliant. A dry membrane is more susceptible to distension due to volume changes and may break if volume is added directly into the cell compartment without wetting of the membrane. Further, the air trapped in the cell compartment is easier removed when the semi-permeable membrane is wet and a medium layer is above.

3.4 Culture Inoculation

The more cells are inoculated, the faster the full capacity of the CELLline bioreactor is reached. In general, INTEGRA recommends a minimum starting inoculum concentration of 1.5×10^6 cells per ml.

Experience shows that in order to compensate for a possible initial lag phase raising the glutamine concentration in the medium to 4 mM can be advantageous. In other cases the increase of FCS in the cell compartment to 20% or of in the medium compartment to 5 % led to an improvement of the initial growth performance.

3.5 Harvesting cycles

The 5 - 7 days harvesting cycle described in the standard protocol is not a fixed period and depends on the cell type used, their viability and individual growth characteristics within CELLline. In general best performances are obtained when the cell compartment is harvested 1 day after reaching maximum cell density. The maximum viable cell capacity in the cell compartment is around 2 to 4×10^7 cells per ml and varies with different cell types.

CELLline gives you a lot of flexibility on establishing your own protocol fitting your individual application, cell type and medium composition below two methods applied:

CELLline classic

When working with suspension cells in CELLline *classic* the standard protocol recommends to reinoculate the cell compartment by a 1 to 4, cell suspension to fresh complete medium split back. With this method the full capacity of the reactor is reached after 5 to 7 days and the nutrient medium should be change on every harvest.

Alternatively, upon harvest the entire cell compartment volume can be harvested and centrifuged. The product-containing supernatant is kept for further processing and cells are counted and reseeded into the CELLline Bioreactor at the around 2×10^6 cells per ml in fresh complete medium. Full capacity of the reactor is expected to be reached after 5 to 7 days and the nutrient medium should be change on every harvest. This method is only recommended with cell types with standing centrifugation.

In other protocols, the cell compartment is reinoculated by a 1 to 1 cell suspension to fresh complete medium split back and with the cell compartment harvested every 3 day. The medium is changed on every harvest (3 day cycles) leading to high product yields or only on every 2nd harvest (6 day cycle) which is the more cost efficient protocol.

3.6 Cell Harvest

The dry semi-permeable membrane is only 8 μm thick. The membrane is delicate but easily withstands normal handling. "Shaking" or "banging" of the flask against hand or surface can lead to membrane failure. Therefore empty the medium compartment gently without shaking the CELLine Bioreactor and do not pipette liquid from or into cell compartment with force. When using an automatic pipetting aid like the INTEGRA Pipetboy set it to lowest power level otherwise aspirate very slowly and fill in by gravity flow.

When working with CELLine *adhere* avoid vigorous manipulations when harvesting of the cells, because this can cause viable cells to detach from the PET inlay matrix. Thus by consequence the cells need more time to reach the full capacity again. Which in turn would diminish the performance of CELLine.

3.7 Osmotic flux

The protein gradient across the semi-permeable membrane can drive water from the medium into the cell compartment. This causes dilution of growth factors which can influence proliferation of the cells. It is possible to compensate for this dilution by adding a slight excess of serum or growth promoting factors into the cell compartment during culture to maintain a satisfactory concentration during culture.

Evaporative loss from the medium compartment will counteract osmotic volume increases and will vary depending upon incubator and frequency with which door of incubator is opened. The user should monitor cell compartment volume during harvests and adjust cell compartment serum levels as required for the individual culture conditions. If a stable cell compartment volume is required, elimination of the protein gradient is required. This may be accomplished by supplementation of inexpensive protein to the medium compartment.