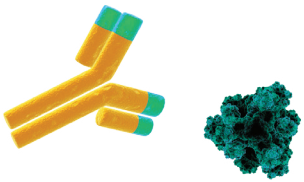
WHEATON®

CELLine™ Bioreactors

Quick Starting the CELLine 350



OBJECTIVE: Get at least 135 x 10^6 viable cells in the cell compartment.

OVERVIEW: Cells and 5mL of medium goes into the port with the white cap (the Cell Compartment). Standard nutrient medium (350mL) goes into the port with the green cap (the Medium Compartment). Pre-warm the nutrient medium to incubator temperature prior to introducing it into the device to minimize condensation and prolonged exposure of the cells to temperatures less than incubator temperature.

HOW TO INOCULATE: First, put about 15mL of medium into the Medium Compartment to make the dialysis membrane compliant. Then, with the green cap slightly loosened, inoculate the 5mL cell suspension into the Cell Compartment using a 10mL pipette. Whenever liquid is added or removed from the Cell Compartment, loosen the green Medium Compartment cap first (it prevents pressure in the Medium Compartment from slowing liquid transfer to and from the Cell Compartment). Remove any large bubbles that are in the Cell Compartment by tilting the device as needed to draw them into the pipette. Tighten the white cap. Next, place the remaining 335mL (i.e. 335 + 15 = 350) of nutrient medium into the Medium Compartment and tighten the green cap. Place the device into a standard CO2 incubator.

GENERAL FEEDING FREQUENCY: As a general rule, 350mL of nutrient medium will support the cells as they expand from about 33 x 10^6 to 135 x 10^6. This typically occurs over a three-day period. Thus, many customers do a complete medium replacement, and cell reduction to about 33 x 10^6, every three days. NOTE: Some customers prefer to only replace the medium once a week in order to save labor and medium costs. They reduce the cells to about 10 x10^6 viable cells at each feeding period. However a 20% to 30% reduction in cell secreted protein can be expected over a 30 day period when feeding occurs only once per week.

GENERAL INOCULATION AND HARVESTING STRATEGIES: The more cells in the cell compartment, the faster production will occur. Therefore, inoculate with as many cells as convenient. Inoculation volume should be 5mL. Once there are at least 135 x 10^6 viable cells in the CL350, the harvest schedule can begin. When to assess the cell compartment to determine the number of live cells depends on how many cells were used for inoculation. Typically, cell number doubles at nearly the rate they do in traditional flasks. Sometimes there is a one day lag before growth begins, so don't be surprised if the initial count hasn't doubled in 24 hours. Thus, if 70 x 10^6 hybridoma cells were inoculated, about 48-72 hours later 140 x 10^6 will reside in the cell compartment. To assess the number of cells, pipette the volume up and down 3 or 4 times to mix the cells in suspension (loosen the green cap while this takes place). Note the volume of liquid in the cell compartment. There should be about 5mL to 7mL. Remove approximately 0.25mL and count cells and determine viability. If the total number of viable cells does not exceed 135 x 10^6, do nothing and check again the next day.

3-Day Feeding Schedule: When the total number of viable cells exceeds 135 x 10^6, harvest by leaving 1.5mL of the mixed suspension in the cell compartment and removing the remaining volume for supernatant. Add enough of fresh medium to restore the cell compartment to 5mL. Replace the nutrient medium in the Medium Compartment. Repeat every three or four days.

5-Day Feeding Schedule: Inoculate (or at steady state split the cells back to) about 42 x 10^6 viable cells and add 350mL of nutrient medium. Three days later, reduce the cell number to about 65 x 10^6 and do not change the nutrient medium. Two days later, reduce the cell number to about 42 x 10^6 and completely change the nutrient medium. Repeat the cycle.

7-Day Feeding Schedule: Inoculate (or at steady state split the cells back to) about 8 x 10^6 viable cells and add 350mL of nutrient medium. Seven days later, reduce the cell number to about 8 x 10^6 and completely change the nutrient medium. Repeat the cycle.

TIPS:

- The color of the basal medium will not change during culture. Therefore, medium exchange must be determined based on the number of cells in the device. As a rule of thumb, 135 x 10^6 cells will consume 350mL of basal medium every three days. Therefore, reduce the cell number as needed to match your feeding frequency.
- We have learned that optimal Mab production is attained when cell viability is at 50% and 60% during each harvest. Do not be concerned, as this has not been shown to affect the quality of the protein in any way.
- If the prior method of culturing the cells used FBS, add 15% FBS to the cell compartment medium and do not use any FBS in the basal medium. If cells are not growing during the first week after inoculation, do not harvest. Replace the basal medium entirely with medium supplemented at 5% FBS. After viable cells reach 135 x 10^6, remove FBS from the basal medium. Very rarely do hybridoma, CHO, or BHK cells need to have FBS in the basal medium.
- The more viable cells in the cell compartment, the better. The first week of culture is generally less productive than following weeks because there are fewer cells. Inoculating with as many cells as possible will make the first week of culture more productive. Use common sense when feeding and harvesting. Do not wait 7 days to feed and harvest the culture if the culture was inoculated at high density.
- Once cells reach 135 x 10^6, counting the cells every harvest is not necessary. However, periodic counts should be made to verify nothing has gone wrong.
- In Mab applications, plan for 10 to 70 mg's of Mab to be obtained in a 30 day period when the three day harvest and feeding protocol is followed. The actual output is wide ranging because the secretion rate of hybridoma clones can vary widely. For example, a clone secreting 30 micrograms per mL will typically produce at least one mg per mL in CELLine.
- Since there is going to be a large amount of debris in the supernatant even after centrifugation, do not pre-filter supernatant prior to purification with a filter that has a porosity less than 0.8 microns. Instead, use a 0.8 micron filter to remove the debris, for example a serum filter, prior to any further filtration.
- Keep the green cap loose when adding and removing liquid to the Cell Compartment. It prevents pressure in the Medium Compartment from occurring, which will slow liquid movement into and out of the Cell Compartment.
- Pre-warm medium before filling the Medium Compartment. It prevents condensation and prolonged exposure of the cells to low temperature medium.