Guide for Antibody Production in a Corning® CELLine™ Disposable Bioreactor

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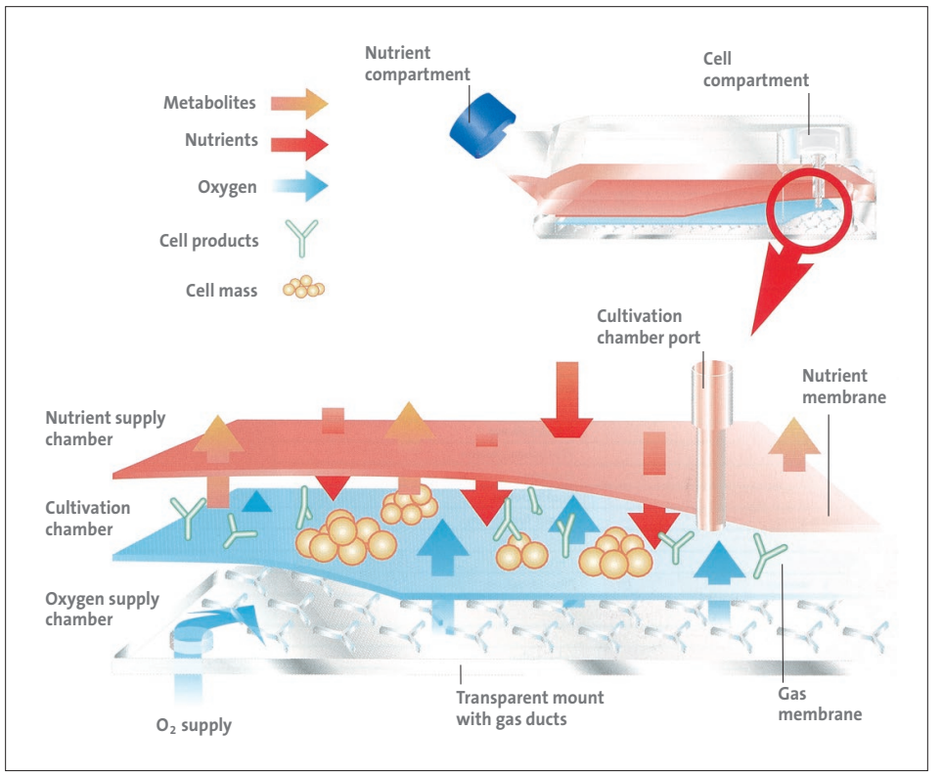
INTRODUCTION

The Corning CELLine Disposable Bioreactor is a novel, multi-chamber cell cultivation system based on membrane technology. This system is easy to use and supports high cell densities, making it ideal for high-yield monoclonal antibody production and recombinant protein expression.

Monoclonal antibodies (MAbs) have become increasingly important as research tools. The primary methods available to generate research quantities of MAbs (10 to 500 mg) are static tissue culture, spinner or roller systems, and ascites fluid from mice. As the demand for MAbs has increased, there has been an emerging need for alternative *in vitro* production methods that minimizes animal use, simplify downstream processing, and reduce variability in production runs. The effort to identify a production method that meets these requirements culminated in the development of the CELLine disposable bioreactor.

When used in conjunction with optimized medium, the CELLine disposable bioreactor generates antibody concentrations comparable to those derived from ascitic fluid. Moreover, the amount of antibody produced in one CELLine disposable bioreactor is equivalent to that derived from 12 mice. The harvest volumes result in antibody concentrations that are 50 to 100 times higher than roller bottles and tissue culture flasks. A typical preparation of monoclonal antibody will range in concentration from 1 to 5 mg/mL.



CELLine Membrane Technology

Cells are maintained in a 15 mL cultivation chamber that is separated from a one-liter nutrient supply compartment by a semi-permeable membrane. Nutrients and other small molecules pass across the membrane into the cell cultivation chamber. Cell-secreted products with a molecular weight greater than 10,000 Daltons are retained in the cell growth chamber of the device. A molded silicone membrane on the bottom of the device allows oxygen to reach the cells from underneath. The cells settle upon the silicone membrane at the bottom of the cell compartment, which provides direct access to oxygen and carbon dioxide gases that rapidly diffuse across the membrane. This approach leads to high cell concentrations within the small volume of medium in the cell cultivation chamber.

Separate ports provide selective access to the nutrient supply chamber and the cultivation chamber. The solid white cap identifies the cultivation chamber port while a blue vent cap identifies the nutrient supply chamber. The cell chamber is accessed via the cultivation chamber port using a serological pipet.

QUICK GUIDE FOR ANTIBODY PRODUCTION

Protocol Overview

Two harvesting regimes are available for production of antibodies using the Corning® CELLine™ disposable bioreactor. The 7/21 day procedure is recommended for continuous harvesting over a period of time. When greater than 50 mg of antibody are desired, the 7/21 day procedure provides the best results.

The 14/14 day procedure is a batch culture method recommended for a one-time harvest. Depending on the particular cell line, the 14/14 day procedure is ideal for generating a single lot of antibody in the 20 to 50 mg range.

Protocol

1. Follow standard culturing protocols to prepare cells for introduction into the CELLine disposable bioreactor.

2. Prepare one liter of culture medium. Use this medium for resuspending the cells and for filling the nutrient compartment. Pre-warm the nutrient medium to 37°C to prevent condensation and to eliminate significant temperature fluctuations inside the incubator.

   Important Note: For serum-supplemented Medium, include 20 to 40% serum in the 15 mL of medium that will be used to seed the cell compartment. The serum concentration in the cell compartment should be roughly double the standard serum concentration. The serum concentration is high to compensate for osmotic flux from the nutrient compartment. The nutrient compartment does not require serum supplementation.

3. Pre-wet the nutrient compartment with 25 mL of culture medium.

4. Count cells and resuspend at desired seeding density in 15 mL of culture medium. If serum-supplemented medium is used, remember to supplement with serum as in step 2. Serum-free formulations do not require serum supplementation. Inoculate cell mixture into the cell compartment using a serological pipet. The blue cap on the nutrient compartment should be loose during inoculation. Remove bubbles from the cell compartment using a serological pipet. When finished, tighten the white cap of cell compartment.

5. Fill the nutrient compartment with approximately one liter of culture medium. Tighten the blue cap after filling the nutrient compartment.

6. Incubate cells in the CELLine disposable bioreactor in a CO_2 incubator at 37°C for 7 to 14 days. All of the antibody that is produced will be concentrated in the white-capped cell compartment.

   Important Note: If your cells have a doubling rate of less than 24 hours is may be necessary to harvest your cells prior to day 7 and reseed them at a lower density in the cell culture compartment. The nutrient compartment can be left alone.

7. Antibody Harvest

   a. 7/21 Day Harvest Procedure

   On day seven, collect the cells and antibody-containing medium from the cell compartment. Make sure the blue nutrient compartment cap is loose as you aspirate the cells and medium from the white-capped cell compartment with a serological pipet. Centrifuge the sample and collect the antibody-containing supernatant. Purify the antibody using established methods.

   Resuspend the cell pellet using the appropriate optimized culture medium and count the live cells prior to reseeding. Reseed the cells into the bioreactor at desired concentration in culture medium and culture for an additional seven days. If necessary, remove bubbles from the cell compartment using a serological pipet. Following the third antibody harvest on day 21, the cells can be maintained for longer periods of time if desired. Medium in the nutrient compartment must be changed at this time. Pour off the spent nutrient medium. Repeat the reseeding process as described above. Add approximately one liter of fresh optimized culture medium supplemented with glutamine to the nutrient compartment. Follow this procedure until sufficient antibody has been obtained.

Cells can be monitored for cell growth and antibody production by periodically removing a small sample from the cell compartment.

b. 14/14 day Harvest Procedure

For a one-time harvest on day 14, collect the cells and medium from the cell compartment using a serological pipet. Centrifuge the sample and collect the antibody-containing supernatant. Purify the antibody using established methods. Discard the 1000 mL of nutrient medium, cell pellet, and Corning® CELLine™ disposable bioreactor.

Important Corning CELLine Disposable Bioreactor Handling Tips

- Prior to adding or removing liquid from the cell compartment (white cap), loosen the blue nutrient compartment cap to prevent air lock.
- Pre-wet the cell cultivation chamber membrane by adding 25 mL media to the nutrient chamber (blue cap) before seeding. This ensures membrane pliability.
- A serological pipet is recommended for cell cultivation chamber (white cap) manipulations. Press the pipet against the black gasket to access the cell cultivation chamber. The gasket will seal around the end of the pipet and allow for pipetting. The pipet does not extend into the cell cultivation chamber on the bioreactor bottom.
- When culturing, there should be no bubbles in the cell cultivation chamber. Bubbles can be removed by tilting the bioreactor and aspirating with a serological pipet pressed to the gasket.
- Do not shake the bioreactor to dislodge cells; this may rupture the cell cultivation chamber membrane.
- When changing the nutrient media (blue cap), pouring is recommended. This gentle method of moving a large volume of medium will preserve the membrane that serves as the top of the cell cultivation chamber.
- Tighten both the blue and white caps before placing the bioreactor into the incubator.

Volumes for the CELLine disposable bioreactor are as follows:

Cat. No.	Description	Cell Compartment	Nutrient Compartment
353137	CELLine Bioreactor	15 mL	1000 mL

FREQUENTLY ASKED QUESTIONS

1. **Why is the removal or addition of media from the cell compartment slow? Why does cell compartment volume come out of the port when I remove the pipet?**

   Be sure that the blue cap is loosened during manipulation of the cell compartment volume. Changes in cell compartment volume create pressure in the nutrient compartment if the blue cap is not loosened. Tighten blue cap after cell compartment manipulation is complete.

2. **I cannot view cells in the bioreactor using my inverted microscope. What can I do to view them?**

   The microscope objective must travel above the stage of the microscope to allow viewing into the cell compartment. If you have a mechanical articulated stage or other attachments on the microscope, you may have to remove them. Alternatively, the objective can be loosened several turns to allow sufficient travel up past the plane of the stage. Additionally, the working distance of the objective must be sufficient; most 10X and some 20X objectives are suitable.

3. **I have viewed my cells in the bioreactor, but when I tried to view them again after several days of culture, I was not able to focus on them. What has changed?**

   When the bioreactor is removed from the incubator and the temperature of air in it is cooled slightly, contraction of the air takes place and draws the cell compartment membrane up into the bioreactor. This contraction lifts the bottom membrane and takes it out of focusing distance. Loosening the blue cap equilibrates pressure and returns the membranes to their original positions.

4. **When I pour medium from the bioreactor, I sometimes have a drop of medium left on the outside of the neck. What can I do to stop this from happening?**

   When pouring medium from the bioreactor, hold the bottom of the bioreactor in the palm. This provides adequate neck pouring angle and prevents accumulation of the medium on the lip of the neck after pouring.

5. **Why is the outside of the bioreactor wet after incubation?**

   Condensation occurs when liquid is placed in a bioreactor that is not pre-warmed. Due to the large volume of medium contained in the bioreactor, condensation can be significant and takes time to evaporate. Test the color of the liquid by blotting with white paper. If there is no coloration, the liquid is water resulting from condensation.

6. **How strong is the semi-permeable membrane?**

   While the upper semi-permeable membrane is only 8 µm thick when dry, it easily withstands normal handling. However, shaking or banging the bioreactor when it contains liquid can significantly stress the membrane due to rapid liquid displacement and should be avoided.

7. **Why is it important to wet the membrane before placing cells into the cell compartment?**

   The wet membrane is compliant and capable of distension. The dry membrane is more susceptible to tensile stress due to volume changes. The air trapped in the cell compartment cannot be removed until the membrane is wet and liquid is added into the cell compartment.

8. **Can I place more than the recommended volumes into the cell compartment?**

   The protocol recommends a working volume of 15 mL. This assures that volume in the cell compartment never exceeds bursting threshold for the membrane, even with osmotic flux of water into the cell compartment over an extended period.

9. **Will I be able to recover all of my cells from the cell compartment?**

   The recovery of murine hybridoma cells from the cell compartment should be nearly 100%. These cell types have not been observed to form aggregates and are readily dispersed with gentle pipetting. Following pipetting, the cells are easily recovered. An additional rinse of clean medium may be used to further assure complete cell recovery, if required. In the recommended protocol, rinsing the cell compartment is not required. Other cell types may form cellular aggregates or attach to the bottom of the cell compartment. In these cases, cell recovery may require the use of a disassociating agent to separate cells and to aid in their recovery.

10. **Are there any tips on handling to reduce the risk of contamination?**

    Every time the culture is handled, the sterile field is broken. Cleaning surfaces with alcohol can provide additional protection against contamination risk. Sterile alcohol wipes or spray alcohol are routinely used to reduce contamination risk in many laboratories. There has been no indication that this adversely affects the bioreactor or cell growth within the bioreactors. If desired, a reduced handling protocol can be evaluated. It is recommended that the individual customer evaluate variation in protocol if reduced handling is desired.

11. When I harvest from the cell compartment, I always have a greater volume than the initial inoculate. Why is this happening?

Osmotic gradients across the upper semi-permeable membrane drive water through the membrane. If a protein gradient is present across the semi-permeable membrane, e.g., when no serum is used in the nutrient compartment, water is driven into the cell compartment. Because small solutes also move across the membrane, this change in volume only affects colloidal protein concentrations. Increased serum is recommended in the cell compartment when no serum is used in the nutrient medium to assure that serum concentrations within the cell compartment do not become excessively diluted with continued culture.

12. Are there any special storage conditions required for unopened bioreactors?

Robust packaging assures that the bioreactors can be stored at ambient conditions with no demonstrated deterioration in performance. Care should be taken to prevent the bioreactors from being exposed to temperatures in excess of 48°C to prevent dimensional changes in the membrane and excessive tensile stress.

13. How much nutrient medium can I place in the nutrient reservoir?

The maximum capacity for the bioreactor is 1000 mL. Do not exceed this volume, as the design requires that there be an air passage to the blue cap. If excess medium is placed in the bioreactor, an air lock can be created within the reservoir compartment.

14. I notice that sometimes the cells are not evenly distributed across the bottom of the cell compartment. Should I mix the cell compartment to provide a more even distribution?

This is not necessary. Experiments that involved resuspending cells in the cell compartment did not lead to increased cell numbers or antibody production. However, an excessive accumulation of cells in one area of the bioreactor, due to a non-level incubator, should be dispersed and allowed to resettle.

15. Can I culture adherent cells in the Corning® CELLine™ disposable bioreactor?

At this time, cultivation of adherent cells is experimental. The silicone bottom of the bioreactor does not support cell attachment or spreading. Many adherent cell lines will not adhere to the bottom of the cell compartment, however certain aggressive cell lines will.

The formation of a monolayer across the bottom of the cell compartment does not utilize the advantages of the CELLine disposable bioreactor regarding high cell density, as the number of cells supported as a monolayer is restricted by the planar surface area. Certain production cell lines, such as CHO and BHK, can be converted to grow in suspension. These will grow to high density when placed in the cell compartment. HeLa cells will also grow to high cell density in the bioreactor. A strictly adherent dependent cell will require a suitable substrate for cell attachment if it is to be cultivated in the bioreactor.

Microcarriers allow cultivation of adherent cells within the bioreactor. It is critical that cells are attached to the microcarriers to assure proliferation. It is possible to place cells and beads into the cell compartment and allow cell attachment to take place. Standing the bioreactor on end and allowing the cells and beads to settle assures cell-to-bead contact and facilitates cell attachment to the microcarrier. Gentle mixing every 30 minutes allows the cell/bead suspension to resettle and assures even cell attachment. The seeding procedure should be done over a two- to three-hour period with 30-minute intervals between dispersion. Cell attachment can be tracked by counting cells that have not attached to the beads and remain in suspension. After seeding, the CELLine disposable bioreactor should be used according to the recommended guidelines. Cells that have seeded onto the microcarriers will proliferate over the entire bead surface. Therefore, significant cell numbers can be obtained in the cell compartment.

16. **Will my hybridoma stop secreting after prolonged culture in the Corning® CELLine™ disposable bioreactor?**

    We have no data that indicates selection of non-secreting clones takes place during culture in the bioreactor, even for low antibody secreting cells. A low secretor can be tolerated due to the increase in product concentration achieved with the bioreactor. In many instances, cells that have very low production capacity can be salvaged by culturing in the CELLine disposable bioreactor to effectively concentrate the secreted product in a small volume of supernatant.

17. **Is the antibody produced in the CELLine disposable bioreactor equivalent to antibody produced in static culture?**

    Analysis by flow cytometry indicates that antibody produced in the CELLine disposable bioreactor yields equivalent binding per mg (fluorescent profiles) when compared to control antibody cultured in static culture flasks.

18. **How many cells can be cultured in the CELLine disposable bioreactor?**

    For a typical murine hybridoma, the viable cell capacity is 450 to 600 x 10^6 cells. If adequate nutrient medium exchange is provided, cell proliferation will continue within the cell compartment even when the maximum viable cell capacity has been reached. This can result in very large numbers of total cells within the bioreactor. In the production of antibody, total cell accumulation is not problematic.

19. **Does the membrane become clogged with use?**

    Performance of the CELLine disposable bioreactors does not decrease with time of culture. This indicates that solute transfer across the membrane does not decrease significantly during culture, and there is thus no significant clogging or fouling of the membrane.

20. **The cell compartment volume in my CELLine disposable bioreactor is less than what I inoculated. Where is the volume going?**

    If the bioreactor is used in a non-humidified incubator or warm room, evaporative losses from the cell compartment can lead to reduced volumes. The bioreactor is intended to be used in a standard humidified incubator. If required, it may be worth evaluating restricting the bottom gas vents to reduce evaporation. This should be done experimentally to determine the balance between evaporative loss and adequate gas exchange.

21. **Can I culture leukemic cell lines in the CELLine disposable bioreactor?**

    Yes, cell concentrations of certain lymphoblastic cells can reach nearly twice that achieved for hybridoma cells. Some cell lines may be dependent upon the use of serum on both sides of the semi-permeable membrane and this can be readily examined.

22. **Will the CELLine disposable bioreactor function in a 7.5% CO_2 environment?**

    Yes, cultures in the bioreactor are under the same CO_2 tensions as in static flasks.

23. **Will serum-free medium work in the CELLine disposable bioreactor?**

    Yes, many customers report excellent results using serum-free medium. The serum-free medium is placed on both sides of the semi-permeable membrane in most cases. As the secreted protein is recovered at high concentration from the bioreactor, it is no longer necessary to concentrate culture supernatant to recover antibody. This eliminates much of the interference associated with serum protein during purification. At antibody concentrations in excess of mg/mL, the cell compartment supernatant can be applied directly to an affinity column.

24. **Are different membranes available for the CELLine disposable bioreactor?**

    At this time, only a 10,000 MWCO membrane is available.

25. I have followed the protocol and my cells are not growing. What is wrong?

Check to ensure that the protocol has been followed. The condition of the cells prior to inoculation is critical. Cells should be taken from logarithmic growth and inoculated at the required cell numbers. Be sure the minimum cell numbers for inoculation are present.

Absence of hybridoma cell growth has been seen under certain conditions. Poor growth has been associated with mycoplasma contamination. Mycoplasma accumulates within the cell compartment and may exert effects not seen in static culture flasks. Cells should be treated to eradicate the mycoplasma and robust cell growth should be established.

There may be some cells that are not capable of growth in the absence of serum components that can diffuse across the upper semi-permeable membrane. Increasing serum concentrations in the nutrient medium and/or cell compartment can be evaluated to determine if this is required.

Finally, if insufficient numbers of cells are inoculated, there may be a significant lag phase prior to cell proliferation. It is recommended that a new inoculum with higher numbers of cells be placed in the cell compartment and results evaluated. If cells require a conditioning factor that diffuses across the membrane, it may be necessary to start with a small volume in the nutrient medium compartment until cell numbers increase. When cell growth is established, adequate nutrient medium must be added to supply the increasing cell mass.

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At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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