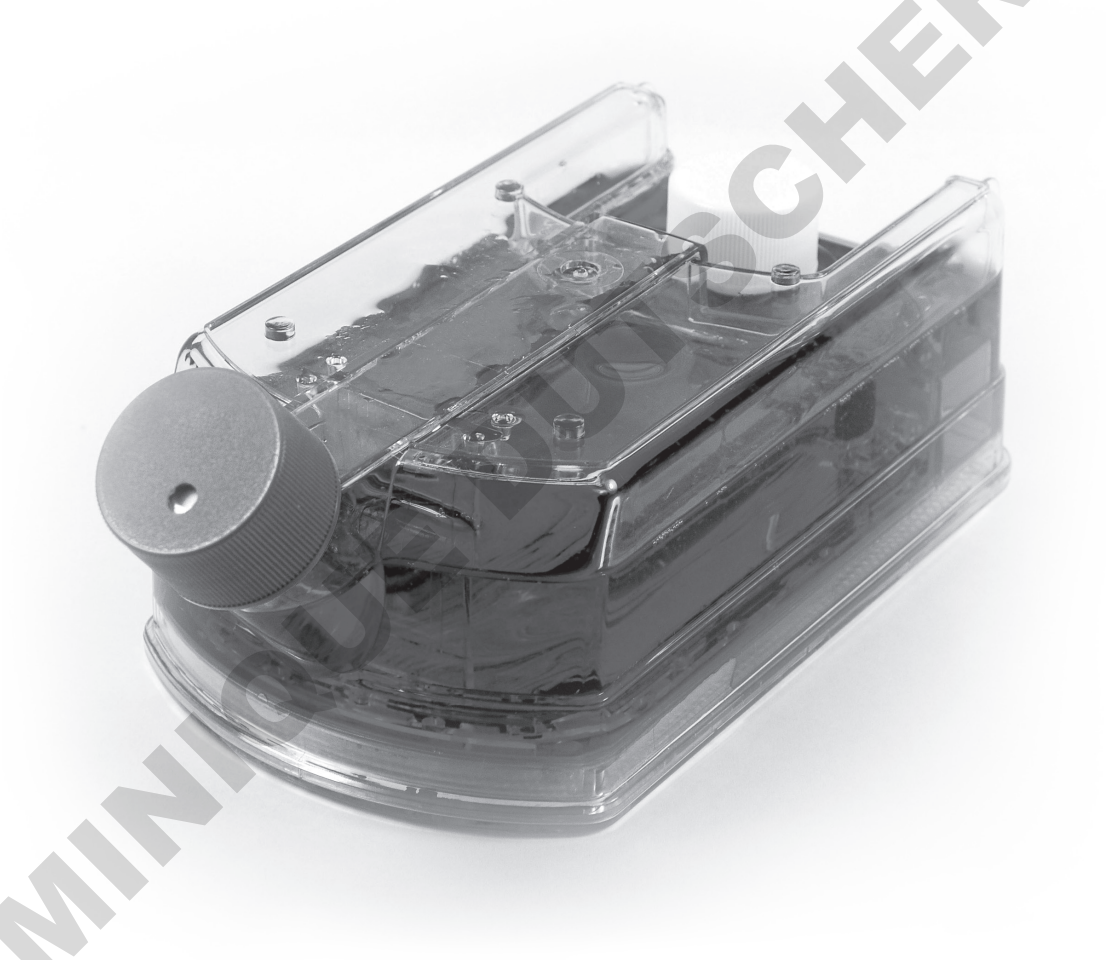
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Advanced Cell Cultivation Devices

User Manual



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CELLine 350: WCL0350-1, WCL0350-5
CELLine 1000: WCL1000-1, WCL1000-3
CELLine 1000 Adherent: WCL1000AD-1, WCL1000AD-3

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1 Introduction

1.1 CELLine™ Two-Compartment Bioreactor Technology

Efficient cell cultivation is dependent on optimal supply of oxygen and nutrients, as well as an efficient removal of inhibiting metabolic waste products. These factors are limiting maximal cell densities in standard, homogeneous cell culture techniques and thus are unfavorable for achieving high expression levels of proteins.

The Two-Compartment bioreactor CELLine is designed to overcome these limitations (see Fig 1). This is attained by separating the bioreactor into a medium compartment and a cell compartment with a 10 kDa semi-permeable, cellulose acetate membrane. This membrane allows small molecules to diffuse from one compartment to the other, while higher molecular weight molecules secreted by the proliferating cells are retained within the cell compartment. This results in a continuous flow of nutrients into the cell compartment and a concurrent removal of any inhibitory waste products. The cell compartment and the medium compartment are individually accessible, which allows you to supply your cells with fresh medium according to their individual needs.

In addition, the bottom of the cell compartment is built of a gas permeable membrane which ensures an optimal oxygen supply and carbon dioxide exchange of your cell culture. All together, this bioreactor approach allows optimal maintenance of the cells at quasi in vivo cultivation conditions. These conditions enable cell proliferation to very high densities within the cell compartment.



Fig. 1 Schematic View of the CELLine Two-Compartment Technology

1.2. Applications

The CELLine bioreactor is suitable for a wide range of different cell culture applications and is proven to cultivate cells at high densities and to maintain aspecfic cell culture over a long time period. In general, CELLine does not require any specific adaptation of your cell culture techniques or media composition and is suitable for applications based on serum-supplemented or serum free cultures.

Suspension and Anchorage-Dependent Cell Types

The choice of **CELLine** or **CELLine Adherent** enables suspension or anchorage-dependent cells to be grown in the bioreactor.

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CELLine is ideal for laboratory scale applications using suspension cells or adherent cells attached to microcarriers. The unit is optimized for cultivation of hybridomas and many other cell types (e.g. CHO, NSO, SF cells).

CELLine Adherent is specifically adapted to promote growth of anchorage-dependent cells (e.g. HEK, BHK, CHO cells). The bioreactor contains a woven, polyethylene terephtalate (PET) matrix in the cell compartment, providing an ideal surface for cell attachment.

High Density Cell Cultivation
With the two-compartment technology of CELLine, deficiencies in nutrition or oxygenation are problems of the past. Therefore, cell densities inside the cell compartment are usually between 10^7 to 10^8 cells per mL, which is about two magnitudes higher than with conventional static cell culture techniques.

High Product Concentrations
Benefiting from the high cell densities, product concentrations achieved in CELLine are in the range of 1 to 5mg per mL, making the bioreactor most suitable for antibody expression in Hybridomas, protein expression in transfected cell lines, or virus production. Product concentrations retained in the cell compartment of CELLine are in most cases 50-100 times above what is found in static cell culture disposables, such as flasks and roller bottles.

Long-term, Continuous Culture Maintenance
Separate access to the medium compartment of CELLine allows you to continuously feed cells without creating any mechanical stress to the culture. Continuous nutrient delivery and efficient oxygenation of the cells enable the user to perform continuous studies on a specific cell culture, or to benefit from a consistent protein production over several weeks.

2 Standard Operating Instructions



CELLine 350

2.1 CELLine 350

2.1.1 Required Material and Preparation

- CELLine 350 Bioreactor
- Standard 10mL serological pipettes
- Pipetting aid
- Preculture of at least 8 x 10^6 viable cells
- 350mL 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 350

Day 1 Put 10mL of nutrient medium into the medium compartment and let the semi-permeable membrane become wet for a few seconds (see 3.3).

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2.1.3 Preparation of Inoculum

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

2.1.4 Inoculation of CELLine 350

Loosen the green cap of the medium compartment in order to prevent air lock. Aspirate the 5mL cell suspension into a serological pipette, remove the white cap from the cell compartment and inoculate the cell compartment by inserting the pipette into the black silicone septum and dispensing medium.

It is important to minimize the introduction of air bubbles into the cell compartment during seeding. If air gets trapped within the cell compartment, remove the large bubbles by drawing them back into the pipette. Angle the CELLine 350 so that the bubbles rise toward the pipette, making removal easier. Dispense any liquid that is removed back into the cell compartment. Close the cell compartment by completely tightening the cell compartment cap.

After seeding, add an additional 340mL of equilibrated medium into the medium compartment. Completely tighten the medium compartment cap. Place the CELLine 350 into a standard CO_2 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. The primary indicator of proper function is the number of cells in the device. At least 135 x 10^6 live cells are expected in the CELLine 350.

2.1.6 Cell Compartment Harvest and Cell Reduction

Day 7 In general, the first harvest is recommended 7 days after inoculation. For alternative protocols and more information, please refer to general note 3.5.

Avoid shaking the CELLine 350 during this process since that may cause the semi-permeable membrane to be damaged (see 3.6).

Remove the nutrient medium from the medium compartment. Replace the green medium compartment cap, but keep it slightly loosened during manipulation of liquid within the cell compartment.

Gently harvest all liquid from the cell compartment by aspirating contents with a 10mL serological pipette. Slowly pipette the liquid up and down several times to thoroughly mix the cell suspension. The cell compartment will comprise about a 5mL cell suspension with the individual secreted product. Due to osmotic flux of liquid from the medium into the cell compartment, the total volume might be slightly increased (see 3.7).

Take 1mL of mixed cell suspension and add to 4mL fresh complete medium (1:4 Split Back) and gently return the 5mL of cell suspension back into the cell compartment (see 3.5).

Remove any air bubbles as described above. Tighten the white cell compartment cap.

Add 350mL of fresh, preheated nutrient medium to the medium compartment. Tighten the green medium compartment cap and place the CELLine 350 back into the incubator until next harvest.

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2.1.7 Harvesting Cycles

From Day 14 Consecutive harvests can be made approximately every 3 to 7 days (depending on the individual application and cell type used - see 3.5 for alternative protocols).

Periodically, cells can be monitored for growth and production by removing a small sample from the cell compartment.

2.2 CELLine 1000



CELLine 1000

2.2.1 Required Material and Preparation

- CELLine 1000 Bioreactor
- Standard 25mL serological pipettes
- Preculture of at least 25 x 10^6 viable cells
- 1000mL of fresh nutrient medium suitable for your individual cell type and equilibrated to the desired culture temperature (see 3.1)
- 15mL of fresh complete medium

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

2.2.2 Equilibration of CELLine 1000

Day 1 Put 50mL of nutrient medium into the medium compartment and let the semi-permeable membrane become wet for a few seconds (see 3.3).

2.2.3 Preparation of Inoculum

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

2.2.4 Inoculation of CELLine 1000

Loosen the green cap of the medium compartment in order to prevent air lock. Aspirate the 15mL cell suspension into a serological pipette, remove the white cap from the cell compartment, and inoculate the cell compartment by inserting the pipette into the black silicone septum and dispensing the inoculum.

It is important to minimize the introduction of air bubbles into the cell compartment during seeding. If air gets trapped within the cell compartment, remove the large bubbles by drawing them back into the pipette. Angle the CELLine 1000 so that the bubbles rise toward the pipette, making removal easier. Dispense any liquid that is removed back into the cell compartment. Close the cell compartment by completely tightening the cap.

After seeding, add an additional 950mL of equilibrated medium into the medium compartment. Completely tighten the medium compartment cap. Place the CELLine 1000 into a standard CO_2 incubator under culture conditions appropriate for your individual cell type.

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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. The primary indicator of proper function is the number of cells in the device. At least 400 x 10^6 live cells are expected in the CELLine 1000.

2.2.6 Cell Compartment Harvest and Split Back

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

Remove the nutrient medium from the medium compartment. Replace the green medium compartment cap, but keep it slightly loosened during manipulation of liquid within the cell compartment.

Avoid shaking the CELLine 1000 during this process since that may cause the semipermeable membrane to be damaged (see 3.6).

With the green medium compartment cap loosened, remove the white cell compartment cap.

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

Take 3mL of mixed cell suspension and add to 12mL fresh complete medium (1:4 Split Back) and gently return the 15mL of cell suspension back into the cell compartment (see 3.5).

Remove any air bubbles as described above. Tighten the white cell compartment cap.

Add 1000mL of fresh, preheated nutrient medium to the medium compartment. Tighten the medium compartment cap and place the CELLine 1000 back into the incubator until next harvest.

2.2.7 Harvesting Cycles

From Day 14 Consecutive harvests can be made approximately every 3 to 7 days, depending on the individual application and cell type used (see 3.5 for alternative protocols).

Periodically, cells can be monitored for growth and production by removing a small sample from the cell compartment.

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2.3 CELLine 1000 Adherent



2.3.1 Required Material and Preparation

- CELLine 1000 Adherent Bioreactor
- Standard 25mL serological pipettes
- Preculture of at least 25 x 10^6 viable cells
- 1000mL of fresh nutrient medium suitable for your individual cell type and equilibrated to the desired culture temperature (see 3.1)
- 15mL of fresh complete medium (see 3.2)

2.3.2 Equilibration of CELLine 1000 Adherent

Day 1 In order to obtain optimal performance of CELLine 1000 Adherent, put 50mL of nutrient medium into the medium compartment and let the semi-permeable membrane equilibrate for a few seconds (see 3.3).

2.3.3 Preparation of Inoculum

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

2.3.4 Inoculation of CELLine 1000 Adherent

Loosen the green cap of the medium compartment in order to prevent air lock. Aspirate the 15mL cell suspension into a serological pipette, remove the white cell compartment cap and inoculate the cell compartment by inserting the pipette into the black silicone septum.

It is important to minimize the introduction of air bubbles into the cell compartment during seeding. If air gets trapped within the cell compartment, remove the large bubbles by carefully drawing them back into the pipette. Close the cell compartment by completely tightening the cap.

After seeding, add an additional 950mL of equilibrated medium into the medium compartment and tighten the medium compartment cap. Place the CELLine 1000 Adherent into a standard CO_2 incubator under culture conditions appropriate for your individual cell type.

2.3.5 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.

2.3.6 Cell Compartment Harvest and Medium Change

Day 10 In general, the first harvest is recommended 10 days after inoculation (see also 3.5).

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

Avoid shaking the CELLine 1000 Adherent during this process since that may cause the semi-permeable membrane to be damaged (see 3.6).

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With the medium compartment cap loosened, gently harvest all liquid from the cell compartment by aspirating the contents with a 25mL serological pipette. The cell compartment will comprise about a 15mL cell suspension and the individual. Due to osmotic flux of liquid from the medium into the cell compartment, the total volume might be slightly increased (see 3.7).

Gently add 15mL of fresh, preheated complete medium into the cell compartment. Tighten the cell compartment cap.

Add 1000mL of fresh, preheated nutrient medium to the medium compartment. Tighten the medium compartment cap and place the CELLine 1000 Adherent back into the incubator until next harvest.

2.3.7 Harvesting Cycles

From Day 17

Consecutive harvests can be made approximately every 7 days (depending on the individual application and cell type used). 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.

3 General Notes for Optimal Use

3.1 Media Preparation

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

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 provides the user additional flexibility beyond traditional cell culture flasks.

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 semi-permeable membrane of CELLine allows the medium compartment to differ from the cell compartment in medium composition. In the standard protocol, the cell compartment is supplemented with about 15% serum, where as the nutrient medium contains only 1% FCS serum or in most cases is entirely serum free.

Serum Free Media
When using serum free medium formulations, it is recommended to use serum free medium in both the cell compartment and the medium compartment. Some customers have reported success in avoiding the use of expensive serum free medium by using standard medium without serum in the cell compartment and standard medium at 10% serum in the nutrient compartment.

3.3 Media Equilibration

It is important to wet the semi-permeable membrane prior to inoculation to assure that the membrane is compliant. A dry membrane may break during distension when liquid is added directly into the cell compartment without wetting the membrane.

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3.4 Culture Inoculation

The more cells that are inoculated, the faster the full capacity of the CELLine bioreactor is reached. In general, a minimum starting inoculum concentration of 1.5 x 10^6 cells per mL is recommended. Typically, the CELLine 1000 will allow over 400 x 10^6 viable cells, and the CELLine 350 will allow over 135 x 10^6 viable cells, to reside in the cell compartment.

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 serum in the cell compartment to 20%, or in the medium compartment to 5% led to an improvement of the initial growth performance.

3.5 Harvesting Cycles

The 3 - 7 days harvesting cycle described in the standard protocol is just a guideline and depends on the cell type used, the viability, and individual growth characteristics within CELLine. In general, the best secreted protein production is attained when the culture viability is about 50% at the time of harvest.

CELLine gives you a lot of flexibility for establishing your own protocol for your individual application, cell type, and medium composition. Two methods are described below:

CELLine
When working with suspension cells in CELLine, the standard protocol recommends harvesting the cell compartment by a 1 to 4 reduction in cells and complete replacement of the nutrient medium. With this method, the full capacity of the reactor is reached every 3 to 7 days.

Alternatively, the harvest can occur every third day, with a medium replacement every sixth day. Many different protocols are successful, and can be tailored to your work schedule. In general, the culture viability should be around 50% viability at harvest for optimal protein production.

Other protocol possibilities include harvesting every three days and reinoculating half of the cells, combined with a complete nutrient medium replacement at each harvest. In this case, the objective is also to attain 50% viability at harvest. Thus, the ratio of cell reduction can be varied depending on the growth characteristics of your cells.

3.6 Cell Harvest Techniques

The dry semi-permeable membrane is only 8 microns thick. The membrane is delicate, but easily withstands normal handling. “Shaking” or “banging” of the flask against hand or other surface can lead to membrane failure. Therefore, empty the medium compartment gently without shaking the CELLine bioreactor.

When working with CELLine Adherent, avoid vigorous manipulations when harvesting the cells, because this can cause viable cells to detach from the PET inlay matrix. Setting the vacuum of the pipette aid to a low value can minimize the amount of cells removed from the cell compartment.

3.7 Osmotic Flux

The protein gradient across the semi-permeable membrane can drive water from the medium compartment 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. For example, when the traditional culture method relies upon a 10% FBS concentration, we recommend a 15% concentration in the cell compartment.

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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 supplementing the medium compartment with inexpensive protein.

4 Troubleshooting and FAQs

1. **Why is it that removal or addition of cell compartment volume is slow? Why does liquid come back out of the cell compartment when I remove the pipette?**

   Be sure that the medium compartment cap is loosened during manipulation of the cell compartment volume. Changes in cell compartment volume create pressure in the medium compartment if the cap is not loosened. An increase in medium compartment pressure tends to drive medium out fo the cell compartment. Loosening the medium compartment cap prevents this condition. Tighten the medium cap after cell compartment manipulation is complete.

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

   When pouring medium from the CELLine, it is recommended that the flask positioned upside down. This provides adequate neck pouring angle and prevents accumulation of the medium on lip of neck after pouring. If a drop of medium does appear on the neck, draw it away with a pipette. If you attempt to remove with an alcohol pad, only sterile alcohol pads should be used.

3. **After incubation, I notice that the outside of the CELLine is wet. Why is this?**

   If medium is placed in flask that is not pre- warmed, there will be considerable condensation accumulation on the outside of the CELLine. Due to the large volume of medium contained in the CELLine, this condensation can be significant. The condensate takes time to evaporate in the humidified incubator. To verify that the wetness is not from leak, verify that the liquid is colorless by blotting with white paper. If there is no color, the liquid is water is due to condensate.

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

   The protocol recommends a working volume of 5mL (CELLine 350) and 15mL (CELLine 1000 or CELLine 1000 Adherent) for the CELLine products. 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. The membrane is fragile, but compliant, and will distend significant distance when wet. Increased cell compartment volumes up to 1.3 times recommendation are not problematic.

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

   When working with cells growing in suspension in CELLine, the recovery of cells from the cell compartment should be nearly 100%. Suspension cell types have not been observed to form aggregates and are readily disbursed 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.

   When working with anchorage-dependent cells in CELLine Adherent, cells are attached to the PET inlay matrix. Depending on the individual cell type, sometimes recovery of the cells can be achieved mechanically by pipetting up and down, but in most of the cases a complete recovery involves the addition of a dissociating agent into the cell compartment.

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6. Can I change nutrient medium by tracking color change of the nutrient medium as I do in my other cultures?

It is not recommended. The medium color change is not an accurate assessment of nutrient and waste status in the CELLine due to the ability of the cell compartment to balance pH directly with the incubator atmosphere. Nutrient medium will become more yellowish during culture, but will not take on the characteristic color associated with spent medium in traditional flasks. Counting the number of cells within the cell compartment can be done to determine optimum medium feed and harvest conditions for your cell type.

7. When I harvest from the cell compartment, I always have a greater volume than what I inoculated. Why is this happening?

Osmotic gradients across the semi-permeable membrane will drive water through the membrane. If a protein gradient is present across the semi-permeable membrane, such as when no serum is used in the nutrient compartment, water is driven into the cell compartment. Because small solutes will move across the membrane also, this change in volume only affects colloid protein concentrations.

This is the reason for the recommended use of 15% serum in the cell compartment when no serum is used in the nutrient medium, as it assures that serum concentrations within the cell compartment do not become excessively diluted with continued culture.

8. The cell compartment volume in my CELLine is less than what I inoculated. Where is volume going?

If the CELLine is used in a non humidified incubator or warm room, evaporative losses from the cell compartment can lead to reduced volumes. The CELLine is intended to be used in a standard humidified incubator.

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

The maximum capacity of the reservoir is marked on the sides of the devices. 350mL is the maximum for CELLine 350 and 1000mL is the maximum for the CELLine 1000 and the CELLine Adherent. Do not exceed these volumes, as the design requires that there be an air passage to the medium compartment cap.

10. I notice that the distribution of cells in the cell compartment sometimes is not even across the bottom of the cell compartment. Should I mix the cell compartment to provide a more even distribution?

This is not necessary. Experiments which involved re-suspending 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 CELLine due to a non-level incubator should be corrected by leveling the CELLine.

11. I have followed the protocol and my cells are not growing, what is wrong?

Try increasing the number of cells during inoculation, adding serum at a 5% concentration to the medium compartment (for serum based application), and verifying that cells are in log phase when collected for inoculation. Contact the distributor or DWK Life Sciences, for assistance. An important aspect of troubleshooting your problem is specific information about the viability and number of cells in the CELLine. Please have that information available.

12. Will the CELLine function in a 7.5% CO_2 environment?

Yes, cultures in the CELLine will be under the same CO_2 tensions as in static flasks. Use of a medium formulated for use in 7.5% CO_2 is required.

13. I cannot view cells in the CELLine using my inverted microscope. What can I do to be able to view them?

Due to PET matrix inlay in CELLine Adherent, cells cannot be viewed under a microscope.

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For CELLine bioreactors, the high density of cells in bioreactor makes viewing difficult. Removing a sample for counts and viewing is recommended.

14. I have viewed my cells in the CELLine 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 CELLine is removed from the incubator and the temperature of air in the CELLine is cooled slightly, contraction of the air takes place and will draw the membranes of the cell compartment up into the device. This contraction lifts the bottom membrane and takes it out of focusing distance. Momentarily loosening the medium compartment cap will equilibrate pressure and return membrane to original position.

15. How strong is the semi-permeable membrane?

The upper semi-permeable membrane is only 8 microns thick. The membrane is delicate, but easily withstands normal handling. Shaking or banging of flask can lead to membrane failure if the cell compartment has liquid in it. Under normal circumstances, the membrane will retain its integrity for over 30 days.

16. Why is it recommended to wet the membrane before placing cells into the cell compartment?

It is important to wet the membrane prior to inoculation to assure that the membrane is complaint. The wet membrane is compliant and capable of distension. The dry membrane is more susceptible to breaking 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. The dry membrane is stressed significantly if volume is added directly into the cell compartment prior to wetting of membrane.

17. Does the semi-permeable membrane become clogged with use?

No, performance of the CELLine devices 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.

18. Are there different membranes available for the CELLine?

Currently, only a 10,000 MWCO membrane is available.

19. Do you have any tips on handling which will reduce the risk of contamination?

The medium is best removed using a vacuum system. If medium is simply poured out, drops on the neck of the bottles should be remove using a sterile pasteur pipette and should not be wiped off with alcohol (unless it is sterile alcohol). It is recommended to perform liquid handling steps with CELLine in a Class II biosafety cabinet.

20. Are there any special storage conditions required for unopened CELLine products?

The devices are packaged in a sterile barrier blister within a foil vapor barrier pouch. Great care has been taken to provide as robust a package as possible. The devices can be stored under ambient conditions with no demonstrated deterioration in performance. Care should be taken to prevent the devices from being exposed to high temperature to avoid dimensional changes in the membrane and excessive tensile stress. It is recommended that devices be stored at room temperature.

21. Will my hybridoma stop secreting after prolonged culture in the CELLine?

We have no data that indicates selection of non-secreting clones takes place during culture in the CELLine. Even for low antibody secreting cells, no evidence has been found indicating that selection for non-secreting cells takes place. Importantly, a low secreting hybridoma will not convert to a high secretor when cultured in the CELLine. However, in many cases, a low secretor can be used due to the increase in product concentration achieved with the CELLine. Thus, CELLine is very useful for cells which have very low production since CELLine allows protein density to increase substantially.

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22. Is there antibody produced in the CELLine equivalent to antibody produced in static culture?

Analysis by flow cytometry indicates that antibody produced in the CELLine yields equivalent binding per mg (fluorescent profiles) when compared to control antibody cultured in static culture flasks recovered. An important CELLine advantage is that excessive amounts of culture supernatant processing is eliminated.

23. How many cells can be cultured in the CELLine?

For a typical murine hybridoma the viable cell concentrations reached in CELLine are around 2-3 x 10^7 cells/mL. If adequate nutrient medium exchange is provided, cell proliferation will continue within the cell compartment even when maximum viable cell capacity has been reached. This can result in very large numbers of total cells within the device. For the optimal production of antibody, total cell accumulation is not problematic and maintenance of 50% viability at harvest is recommended.

24. Can I culture leukemic cell lines in the CELLine?

Lymphoblastic cells grow very well in the CELLine 1000. 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 should examined if optimization is desired.

25. Do you recommend the use of a high glucose containing medium in the CELLine?

Most protocols were developed using standard RPMI-1640 medium. Some customers and others who use hollow fiber bioreactors do use richer mediums. A slight performance increase may be obtained with richer media, however, this is dependent upon cell line and should be evaluated experimentally. In general, excellent results are obtained in medium which is currently used to culture the cell line in static flasks.

26. Will serum free medium work in the CELLine?

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. Importantly, the use of serum free medium may no longer be necessary when the CELLine is used. As the secreted protein is recovered at high concentration from the CELLine, it is no longer necessary to concentrate culture supernatant to recover antibody. This eliminates much of the interference associated with serum protein during purification. Some customers apply the cell compartment supernatant directly to an affinity column.

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5 Literature

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