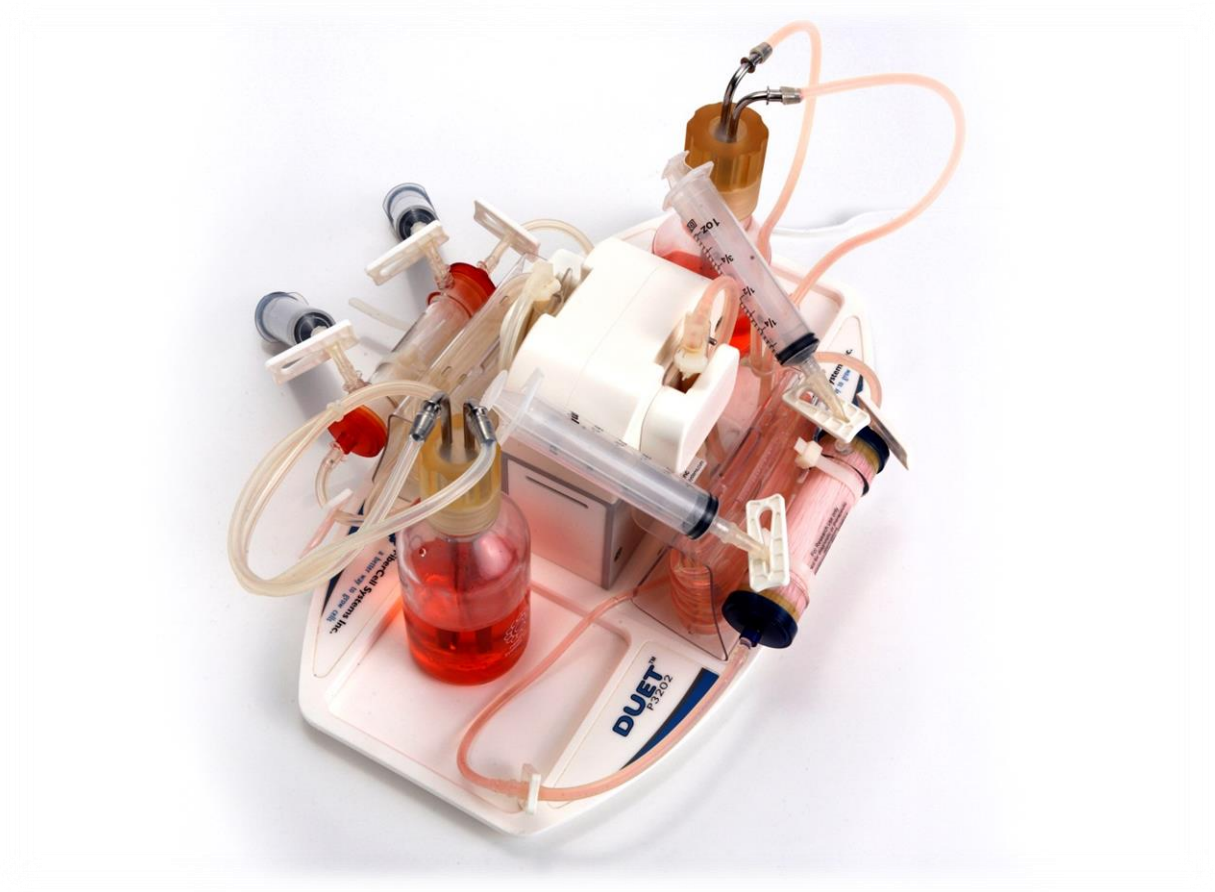


User's Manual

FiberCell Systems®
A BETTER WAY TO GROW CELLS

FiberCell Systems® Inc., A Better Way To Grow Cells
168 West Main Street, #922, New Market, Md. 21774
www.FiberCellSystems.com, To Order Call 301-471-1269 or 240-440-2662
Revision 3.3 2/11/2022

Table of Contents

Duet Operating Instructions

Warning and Safety Instructions

Electrical

- The FiberCell® Systems Duet operates on 24 volt DC. The universal power supply will accept any voltage.
- Do not attempt to service the Transformer, Control Box, or Perfusion Pump.
- Do not place the Transformer or the Control Box inside the incubator.

Cleaning and Disinfecting the Transformer, Control Box and Perfusion Pump

- Wash with mild soap and warm water if needed.
- Never immerse in water.
- Do not use organic solvents or corrosive chemicals to clean or disinfect.
- Do not disinfect by steam sterilization or heat.

Safety

- Always treat the FiberCell® Systems module using biosafety levels recommended for the infectious agents you are handling. After use dispose of according to local Heath Safety Guidelines.
- Always wear lab coats and gloves when handling the FiberCell® Systems module.

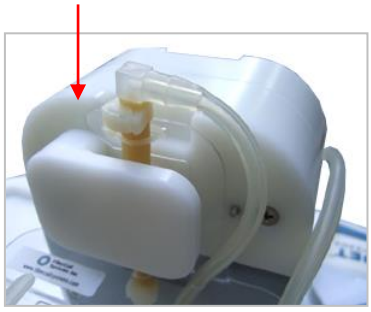
Operation of Duet Pump and Flow Rate Controller

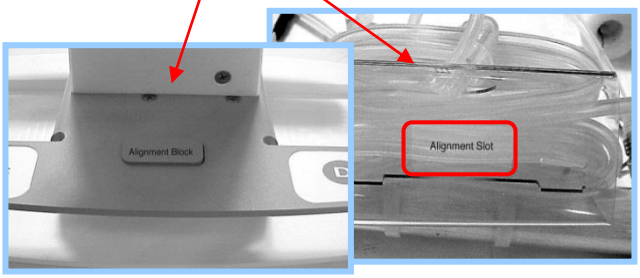
- Insert the Universal Power Cord into the Transformer.
- The Transformer and the Control Box are connected via a standard Ethernet connector. Place these near the incubator in a location where they will be (i) visible and (ii) safe from water spills.
- Do not place on the floor.
- Plug the Power Cord into a grounded outlet. Use of a surge protector is recommended.
- Place the system in the incubator making certain that the white power cord is protected from kinks or abrasion.

The Duet is designed to support culture in one or two of the C2003, C2008, C2011, C2018, C2025, and C5011 cartridges. It will deliver a maximum of 180 mL/min flow rate utilizing a unique positive pressure displacement mechanism that produces no wear on the pump tubing. Low voltage power (24 volts, single phase AC) is delivered to the unit inside the incubator via a thin cord that is designed

to fit through the rubber incubator door gasket. Operation of the duet is quite simple but a few precautions should be noted:

1. Ensure that the brown pump tubing is aligned vertically in the area between the pump piston and pump platen. For maximum flow rate it is essential that this tubing be inserted properly.
2. The cartridge can be most easily inserted and removed from the Duet when the pump is operating. Check to see that the rectangular alignment slot in the bottom of the flow path is inserted ***over*** the alignment block on the base of the Duet. It will click into place. Proper alignment is essential for optimum performance.
3. Up to a 2 liter plastic bottle can be placed into the indentation on the base of the Duet.
4. Flow rate is set by adjusting the knob on the control box. The numbers do not refer to a specific flow rate but to relative positions. A speed of 18 represents the equivalent flow from the maximum setting of older duets, approximately 60mls/minute. Simply turn the knob to adjust flow rate.





Precautions

- ✓ Do not insert fingers or delicate objects between the pump pistons and pump platens.
- ✓ Do not leave the pump inside an incubator unless the motor is running or rust may occur.
- ✓ Do not wipe the unit with anything stronger than 70% ethanol.
- ✓ Do not use bleach or other strong solvents.
- ✓ Do not autoclave the unit.
- ✓ Do try to keep the unit plugged in to a surge protector or battery back-up power source.

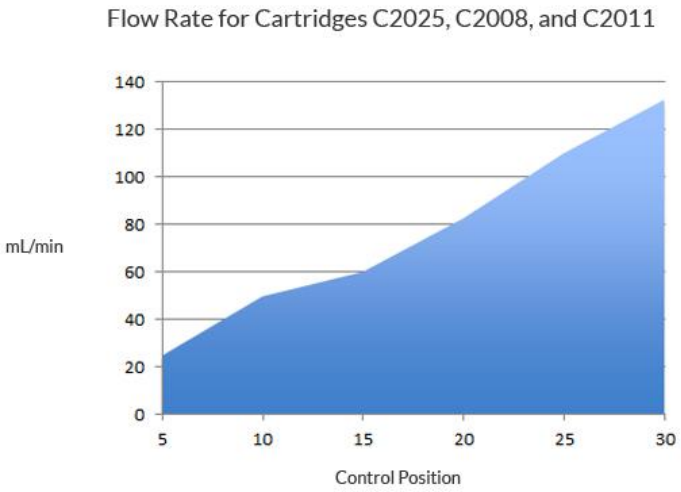
Use of the FiberCell® Systems Pump

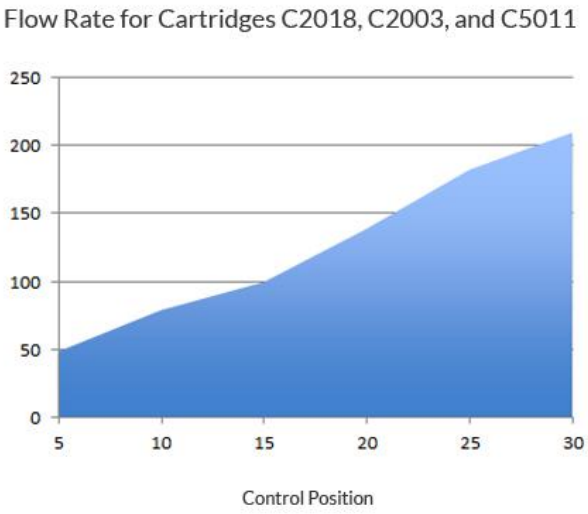
The FiberCell® Systems Duet pump system is designed to operate inside a standard CO_2 incubator. The flat white cord will fit in between the glass door and gasket of the incubator without significant loss of CO_2. The use of a simple surge protector is recommended to protect the microprocessor controller of the pump system.

Recommended Flow Rate Settings

For use with FiberCell® Systems medium sized cartridges (cat# C2008, C2011): **During pre-culture and the first 3 - 4 days of culture set the rate to 18 - 20. After the first 3 - 4 days of culture increase the rate to 25 and leave it set there.**

For use with FiberCell® Systems large cartridges (cat# C2003, C2018): These cartridges generate higher flow rates as they have a larger diameter pump tubing. With the Duet pump initiate culture with the flow rate control knob turned to 18 - 22. After two days, increase to 25 - 30.

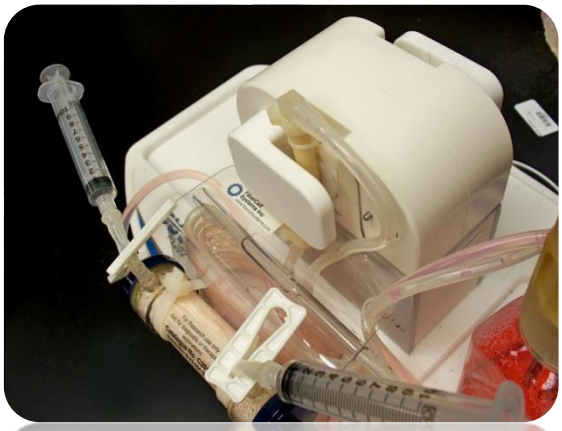




Overview of Hollow Fiber Cell Culture

2

Background of Cell Culture



In vitro culture, or *tissue culture*, of mammalian, insect and plant cells allows the researcher to reduce the multitude of systemic variables that are inherent to the whole organism. The original tissue culture methods were just that, culture of excised tissue and mechanically or enzymatically prepared tissue fragments. The first attempts at tissue culture used the seeding of these cells on top of plasma clots, a porous support. Later these cell lines were cultured in monolayer on glass or treated plastic culture dishes or flasks, a non-porous support. The use of fetal bovine serum played a major role in the development of cell culture methods providing attachment factors and a protein and growth factor rich supplement to the basal mediums of the day. Static culture creates a feast or famine environment with conditions constantly in flux. (1) lag phase while the cells condition the medium, (2) exponential

phase with maximum growth and viability, (3) stationary phase in which medium nutrients are depleted and metabolic activity and viability decreases, and (4) death phase where decreased oxygen, toxic levels of lactic acid, ammonia and decreased pH all contribute to cell death. Prior to this, the cells must be split into new flasks with fresh medium and the cycle continues. There is only a short period of time when cell culture conditions are truly physiologic and optimum.

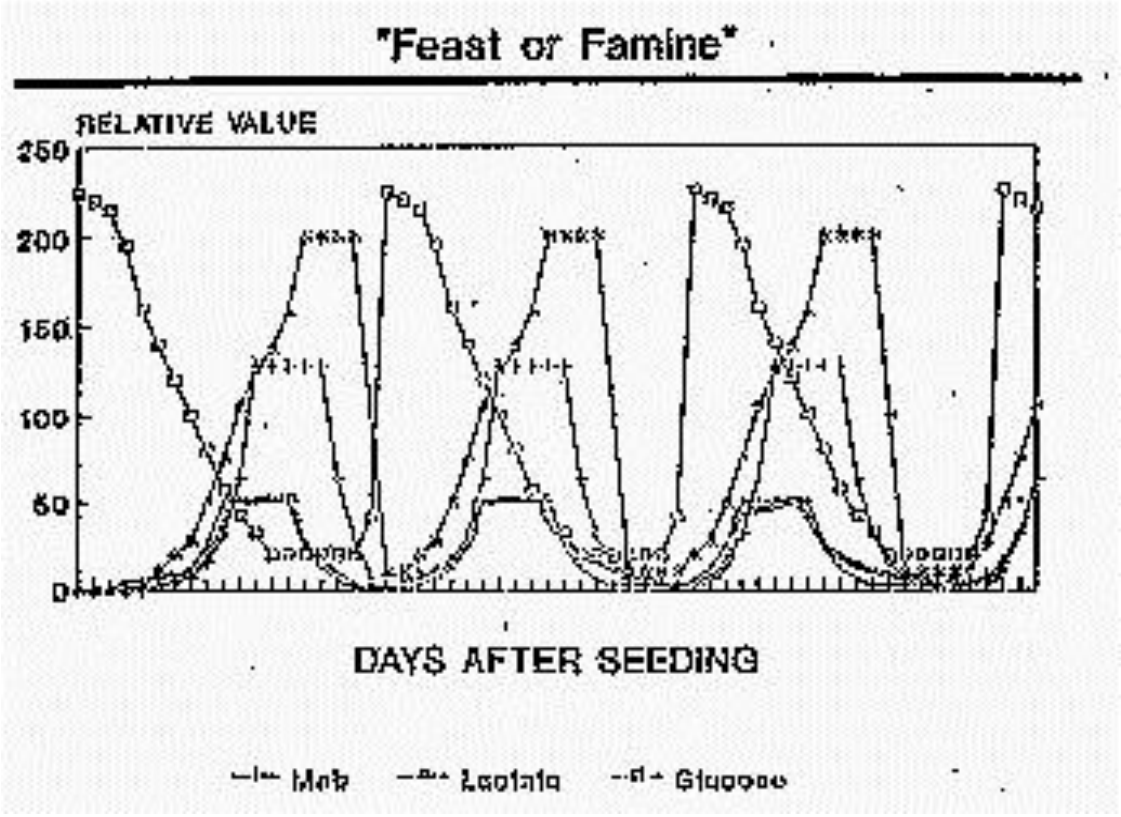


Figure 1. Feast or Famine cycle for hybridoma cells in static flask. Cells exhibit a lag phase after inoculation as they condition the medium. They then enter log phase growth, which is characterized, by glucose consumption and an accumulation of lactic acid and other metabolic waste products. The growth medium becomes acidic as the lactic acid accumulation produced a rapid drop in the pH of the medium.

Classical batch-style 2D cultures on non-porous supports in flasks or low-density suspension culture in spinners or shakers are not biologically relevant models. Cell culture conditions can and do affect the quality and purity of secreted products and how cells behave related to their *in vivo* function.

The hollow fiber bioreactor is a high-density, continuous perfusion culture system that closely approximates the environment in which cells grow *in vivo*. It consists of thousands of semi-permeable hollow fibers in a parallel array within a cartridge shell fitted with inlet and outlet ports. These fiber bundles are potted or sealed at each end so that any liquid entering the ends of the cartridge will necessarily flow through the interior of the fibers. Cells are generally seeded outside the fibers within the cartridge in what is referred to as the extra capillary space or ECS.

Hollow fiber bioreactors (HFBR) contain all of the support systems found in the vertebrate body i.e., the organ (bioreactor), blood (the circulating medium), lung (oxygenation system), capillary bed (hollow fiber dialysis), and heart (pulsatile perfusion pump).

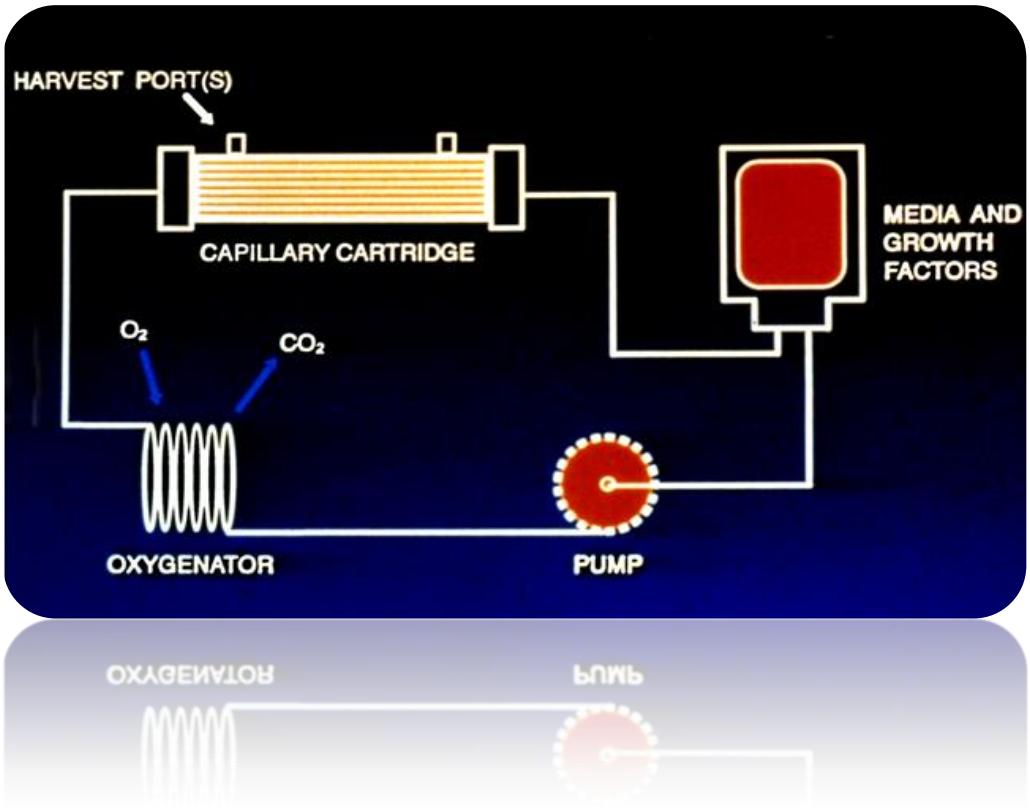
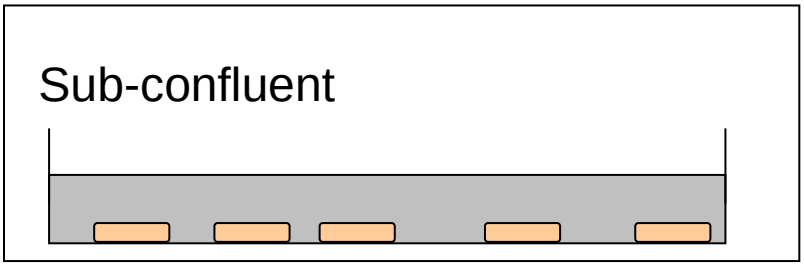


Figure 2. Schematic of a hollow fiber bioreactor. Medium flows from the Reservoir Bottle by means of a pulsatile perfusion pump through the oxygenation circuit where it is oxygenated and saturated with CO_2. Cells growing in the 3-dimensional environment within the bioreactor are bathed in fresh medium as it perfuses through the hollow fibers. Metabolic waste is dialyzed away from the cells and diluted into the circulating medium.



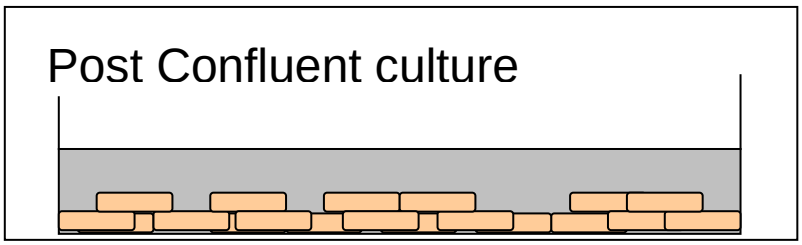


Figure 3a. Static culture in dishes and flasks. After a few days of culture cells are exposed to high levels of lactic acid and other metabolic waste products. Oxygen tension is high at the surface of the medium but low in the microenvironment around the cells. Under static culture conditions metabolic activity and secreted protein production is low. Cells must be split once confluence is reached as the bottom layer of cells do not receive nutrients or logarithmic growth of cells ceases and the cell sheet dies

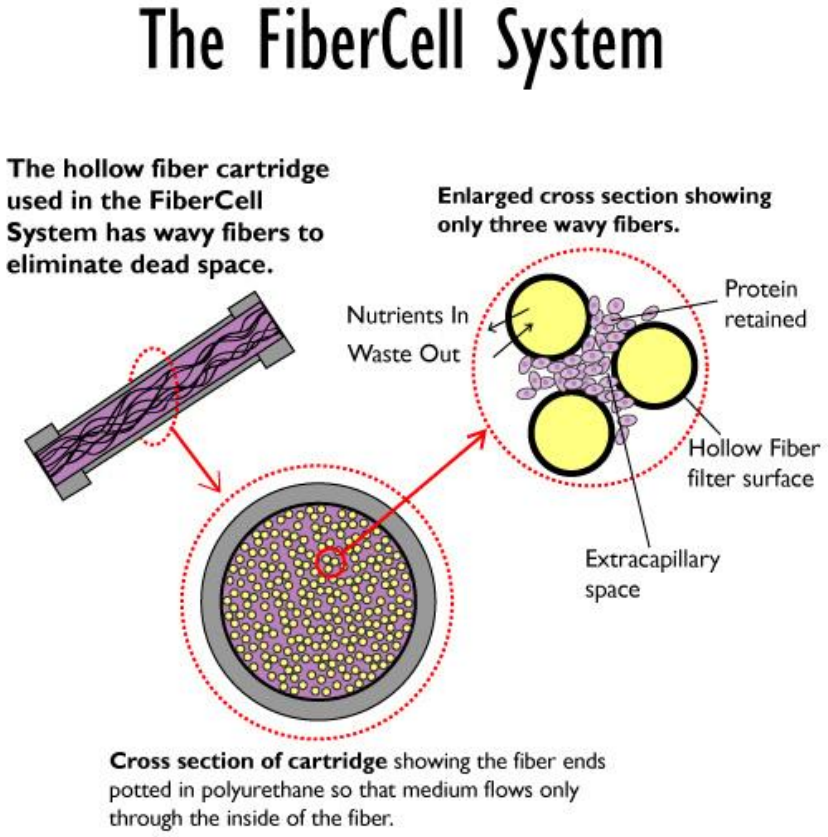


Figure 3b. Perfusion culture of cells in a hollow fiber bioreactor. Medium perfusing through the lumen of the hollow fibers delivers oxygen and nutrients to the cells. At the same time, lactic acid, ammonia and other metabolic waste products are dialyzed away from the cells and diluted into the medium contained in the reservoir bottle. Cells are growing in a 3-dimensional environment, have numerous physical contact points with neighboring cells, and secrete both autocrine and pericrine factors that accumulate within the extra capillary space (ECS).

Culture media is pumped inside the hollow fibers allowing nutrients, gas and waste products to diffuse both ways across the fiber walls. Once having passed through the cartridge, the culture medium is oxygenated and re-circulated to the cartridge. There are three fundamental characteristics that differentiate hollow fiber cell culture from any other method.

1. Cells are bound to a porous matrix much as they are in vivo, not a plastic dish, microcarrier, or other impermeable support.
2. The molecular weight cut off of the support matrix can be controlled.
3. Extremely high surface area to volume ratio. (150 cm^2 or more per mL.)

Cells are bound to a porous support much as they are *in vivo*. There is no requirement to split cells. Cells in this perfusion system maintain viability and production-relevant metabolism in a post-confluent manner for extended periods of time, months or longer. For example, one hybridoma was reported to maintain efficacious productivity for over one year of culture. The more in vivo like growth conditions afforded by HFBRs result in significantly reduced apoptosis (1). The majority of cells that become necrotic will not release cytoplasmic proteins or DNA into the culture medium again resulting in a product, which is cleaner and easier to purify from the bulk harvest.

The molecular weight cut off of the fiber can be controlled. Desired products can be retained to significantly higher concentrations and the effects of cytokines can also be controlled. This is especially important for hybridoma culture in which the inhibitory cytokine TGF beta can be selectively removed from the culture while the secreted antibody is retained.

Extremely high surface area to volume ratio. The small diameter of the fibers (200 microns) generates an extremely high surface area to volume ratio in the range of 100 - 200 cm^2/mL. Coupled with the high gross filtration rate of FiberCell® Systems' polysulfone fibers the exchange of nutrients and waste products is very rapid. Cell densities of 1 - 2 X 10^8 or more are achieved, close to in vivo tissue-like densities. A 20 mL cartridge will support as many cells as a 2-liter spinner flask or 20 - 40 roller bottles. High cell densities produce more protein per milliliter volume and also facilitate adaptation to lower serum concentrations or a simplified protein free serum replacement such as CDM-HD. The use of protein free mediums results in much cleaner harvests of products and simplified purification.

CDM-HD is a protein free, animal component free, chemically defined serum replacement that is optimized for cell culture at high densities, such as is found in hollow fiber bioreactors. It contains specific micro-nutrients, amino acids, free iron, additional buffering capacity and no surfactants as part of its proprietary formulation. It is supplied as a dry powder and is used as a serum replacement for most cell types.

The above features result in protein and antibody concentrations that can be 100 X higher than that found in flask or spinner culture with almost no contaminating proteins from either the cell culture medium or the cells themselves. The more *in vivo* like cell culture conditions can also result in improved protein folding and more uniform glycosylation patterns over time. Since it is a continuous perfusion system the amount of protein produced is determined as much by the length of time the culture is maintained as by such parameters as the clone's specific productivity or the size of the cartridge.

Hollow fiber cell culture is fundamentally different from conventional flask and spinner culture. In summary;

- Cells are bound to a porous support and do not require splitting. Passage number is irrelevant and cultures can be maintained for many months.
- Cells are grown at high densities, typically 1 - 2 X 10^8/mL. It is one of the only methods to grow cells at physiologic densities.
- High cell density facilitates the adaptation to serum free mediums and CDM-HD, a simplified serum replacement optimized for high density cell culture
- Secreted products are concentrated by a factor of up to 100 X
- Post-translational modifications can be more complete, and more *in vivo* like.
- Apoptosis can be significantly reduced resulting in lower contamination with intracellular DNA and proteins
- Inhibitory cytokines can be dialyzed away from the cells.
- Medium does not require surfactant.

Applications

Applications for hollow fibers include:

- Production of secreted products (monoclonal antibodies, recombinant proteins, stable S2 insect lines and conditioned medium)
- Production of high titer virus stocks
- Exosomes
- Expansion of peripheral blood lymphocytes and CD4+, CD8+ PBL subsets and bone marrow stem cells
- Co-cultivation of two cell lines usually consisting of the feeder cells and the expansion cell line.
- Endothelial cell (EC) and EC/smooth muscle (SMC) cultured under conditions of controlled shear stress and pulsatile perfusion
- EC/astrocyte blood brain barrier (BBB) models
- Bio-artificial liver (BAL) devices
- Culture of cell types on specific extra-cellular matrixes
- Pk/Pd studies for in vitro toxicology

The key steps in the use of hollow fiber bioreactors are:

1. **Preparation**
    a. Selection of fiber type, molecular weight cut off (MWCO) and bioreactor size
    b. Assembly of the bioreactor
    c. Pre-culture with growth medium
    d. Expansion of cell line(s) for inoculation
2. **Inoculation**
    a. Harvest cells for inoculation
    b. Inoculation into bioreactor
    c. Cell attachment and adaptation
    d. Medium flow rate ramp up
3. **Expansion**
    a. Monitoring the glucose in the medium

b. Growth rate calculations
c. Correlation between glucose rates and cellular growth rate
d. Feeding and other medium management procedures

4. **Harvest**
   a. Secreted proteins or other products from ECS or reservoir
   b. Suspension cells from ECS

Technical Support

Over 200 different cell lines have been cultured in hollow fiber bioreactors. The general systems and protocols for hollow fiber cell culture have been refined over the last 30 years and have evolved into a straightforward technology. FiberCell® Systems has protocols and application guides for all common applications. Please contact us for assistance with protocol development for your specific application. info@fibercellsystems.com or 301-471-1269.

Criteria for Fiber Selection

Cells cultured in hollow fiber bioreactors exist in a microenvironment where secreted factors accumulate and, in many cases, are feedback regulated. The selection of both the fiber composition and pore size will depend on your application.

- Anchorage dependent cells will attach readily to the polysulfone fiber provided attachment factors or serum are present in the cell inoculum. If particular extra-cellular matrix proteins or surface modifications are desired then the Polysulfone Plus fiber (c2025) should be used.
- If secreted products are desired, the pore size selected will depend on the size of the protein to be produced and whether it can be trapped within the ECS. Proteins greater than 120 kd can be trapped in the ECS of the 20 kd fiber. For proteins between 20 kd and 120 kd the 5 kd fiber should be used.
- Does the cell secrete cytostatic or cytotoxic products? If so, the MWCO should be selected that permits these factors to diffuse away from the cells into the reservoir bottle. Examples of this include Tgf Beta for hybridoma cells (MW 27 kd) and TNF Alpha for lymphocytes (MW 17 kd)
- Is co-cultivation desired? For stromal co-cultivation the 5 kd MWCO is typically used in order to concentrate secreted cytokines. If co-cultivation is in conjunction with endothelial cells the PS+ fiber should be used (catalog #C2025)

Pore Size and Molecular Weight Cutoff (MWCO)

The diffusion characteristics for hollow fibers can be classified into two general categories, i.e., on the basis of either pore size or MWCO.

Pore size is defined on the basis of diffusion coefficients and not on the actual diameter of a geometric "pore" in the wall of the hollow fiber. Typical pore sizes range from 0.1 to 0.5 microns. These fibers allow for very rapid diffusion of oxygen and even the largest proteins. For example: IgM (~600 kDa)

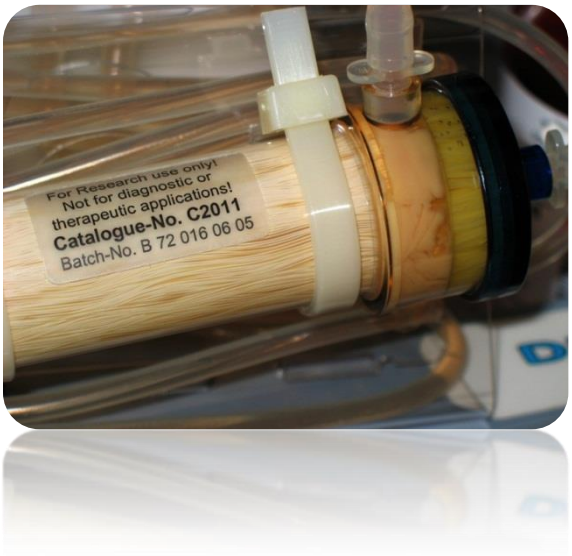
and Blue Dextran (~2 X 10^6 kDa) will readily diffuse across a fiber with a 0.1 micron pore size. Wall thickness in this type of fiber tends to be on the order of 100 to 500 microns.

MWCO specifications are defined using both the 50% and 95% levels depending on the manufacturer. A fiber with a 20 kDa MWCO at 50% will retain ~50% of a secreted protein of 20 kDa within the ECS. The 95% value for this same fiber will be approximately 120 kDa, i.e., it will retain ~95% of a 100 kDa secreted protein within the ECS. If the secreted protein of interest is between 20 kd and 100 kd then the 5kd fiber should be used. For proteins or cytokines smaller than 20 kd utilize the 20 kd fiber and harvest from the reservoir bottle as the higher GFR will result in more rapid cell growth due to more rapid exchange of nutrients and waste products. Do keep in mind that protein size is defined more by the Stokes Radius rather than simple molecular weight and will be affected by glycosylation and shape.

Table I. FiberCell Cartridge Specifications

CATALOG NUMBER	MWCO (@50%)	AREA cm^2	FIBER TYPE	ECS VOL (ml)	GFR#	INOCULATE##
C2025	.1µm	70cm^2	Polysulfone Plus	2	NA	1-5 X 10^6
C2011	20 kDa	3000 cm^2	Polysulfone	20	130	1 X 10^8
C2008	5 kDa	3000 cm^2	Polysulfone	20	12	1 X 10^8
C2018	20 kDa	1.2 m^2	Polysulfone	70	130	4 X 10^8
C2003	5 kDa	1.2 m^2	Polysulfone	70	12	4 X 10^8

NOTES:
GFR#units: mm Hg/cm^2/min
INOCULATE## or a number of adherent cells that will provide ~50% fiber surface confluence.



Cartridge Selection Guide

- **C2011: Protein range 1 kDa - 10 kDa, greater than 100 kDa** High molecular weight cut-off hydrophilic fiber for trapping larger molecules**. Specifically recommended for hybridoma culture, recombinant protein production, virus production and lymphocyte culture**. 20 kd MWCO allows TGF-beta and TNF-alpha to diffuse away while retaining antibody. Will usefully retain proteins greater than 100 kd in size. Proteins smaller than 10 kd can be harvested from the medium reservoir bottle. Can support 1

- 2 X 10^9 cells and produce 5 - 50 mg of monoclonal antibody every two days. 2 - 5 mg of a recombinant protein every day. Also used for medium scale adenovirus production (1 - 5 X 10^{12} pfu). High gross filtration rate makes this fiber ideal for high growth rate cell types. Will consume up to 1 liter of cell culture medium per day.

- **C5011:** Cartridge is the same as C2011 but with 2 X more tubing for oxygenation and larger pump tubing for higher flow rates. This cartridge is intended to be a 2 X scale up for monoclonal antibody production from the C2011. Can produce 10 -100 mg of monoclonal antibody every two days and consume a liter of medium every day.
- **C2018 Protein range 1 kDa - 10 kDa, greater than 100 kDa** High molecular weight cut-off hydrophilic fiber for trapping of larger molecules. **Specifically recommended for hybridoma culture, recombinant protein production, adenovirus production and lymphocyte culture.** 20 kd MWCO allows TGF-beta and TNF-alpha to diffuse away while retaining antibody. Will usefully retain proteins greater than 100 kd in size. Proteins smaller than 10 kd can be harvested from the medium reservoir bottle. Can support up to 5 X 10^{10} cells and produce 5 - 25 mg of recombinant protein every day. Also used for large scale adenovirus production (1 - 5 X 10^{13} pfu).
- **C2008 Protein range 10 kDa - 100 kDa** Low molecular weight cut-off polysulfone fiber for adherent and suspension cell types. Secreted proteins in the range of 20 kd and higher will be retained in the small volume of the ECS (20 mL). Up to 10^9 cells can be cultured. Will consume up to 1 liter of cell culture medium per day.
- **C2003 Protein range 10 kDa - 100 kDa** Low molecular weight cut-off polysulfone fiber for adherent and suspension cell types. Secreted proteins in the range of 50 kd and higher will be retained in the small volume of the ECS. Up to 5 x 10^{10} cells can be cultured. Will consume up to 4 liters of cell culture medium per day.

General Rules for Harvest of Adherent Cells

Enzymatic

- Harvest of cells that have attached to the fibers is usually not possible using trypsin or other enzymatic treatments. Unlike adherent cells cultured in monolayer in dishes, cells cultured in hollow fiber bioreactors form multiple layers and are, therefore, resistant to trypsinization. Good results have been obtained utilizing Accutase or Hyqtase. Adapting the cells to serum free culture, using CDM HD or other serum/protein free mediums will facilitate this process.

Non-Enzymatic

- Non-adherent cells such as lymphocytes exhibit only a slight adherence to the fibers. In most cases, recoveries >90% can be achieved by flushing the cells from the ECS with growth medium.
- With adherent cells in long-term culture, e.g., hybridoma lines and recombinant CHO cell lines, the total cell load within the ECS must be reduced regularly to prevent the ECS from completely filling with cells or exceeding the capacity of the system to deliver oxygen. Utilizing the high glucose rate harvest protocol will remove dead and lightly adhering cells

leaving the more metabolically active cells on the fibers. See the video manual for compete instructions

- If complete recovery of adherent cells is desired, for further expansion or biochemical analysis, the only practical method to recover adherent cells is to cut open the ECS and to remove the fiber bundle. Cells can then be dislodged from the fibers by mechanical scraping or agitation in PBS or serum-free growth medium**.**

Sterile Technique

Contamination is a primary concern with all cell culture and it is essential that good cell culture technique, appropriate precautions and correct protocols be used with hollow fiber cell culture. Use medium that is sterile and always work with the bioreactor module inside the laminar flow hood. The following precautions are recommended.

Never take short cuts.

Module

- After initial set-up and pre-culture, always wipe any fittings or bottles with 70% alcohol swabs before and after they are opened.
- Wipe any spilled medium from the module with an alcohol swab.
- Always use new luer caps after harvesting from the ECS.
- If fungus is a problem in your laboratory use 70% ETOH along with bleach.

Laminar Flow Hood

- Clean the hood with disinfectant or alcohol before and after use.
- Work with only one cell line and medium supply at a time.
- Don't clutter the hood. Keep the laminar airflow pathways clear.
- Wear powder free disposable gloves and lab coats.

Growth Medium

- Use one bottle of medium for each cell line. Do not share bottles with different cell lines.
- Always warm medium in a 37° C degree water bath before opening. This will avoid the potential contamination from air being drawn into a cold bottle of medium upon opening. After removing the bottle from the water bath spray it with 70% alcohol before placing it under the flow hood and opening. Also spray the neck and cap of the bottle with 70% alcohol and wipe prior to opening.

General Protocol for Hollow Fiber Cell Culture

The following specific information and recommendations regarding hollow fiber tissue culture systems may be useful for those of you already comfortable with basic cell culture techniques. This information may be used as a guide in helping you to maximize cell production and protein or virus output. This general protocol should be used in conjunction with the FiberCell® Systems Video Manual. Please read the specific sections of this manual for more detailed information.

Regulating cell density (cell count per mL) is the goal in stabilizing your hollow fiber system. Too low a cell density and your cells will go into stasis (stop growing), too high a cell density and oxygenation becomes rate-limiting resulting in increased anaerobic metabolism and poor cell growth. If the doubling time of your cell line is 48 hours then every 48 hours you should be removing one half of the cell population. You should remove a cell pellet of between 1 - 5 mL for the C2008, C2011 and C5011 and 5 - 20 mL for the C2003 and C2018.

Pre-culture of the system with cell culture medium is an important step and should not be omitted. This equilibrates the system with media, coats charged sites with protein and insures that the system is leak free and sterile prior to the inoculation of cells. Serum is not required in the pre-culture medium until the final change. If anchorage dependant cells are to be used it is critical that the cartridge be pre-cultured with PBS prior to the pre-culture steps outlines above. The fibers are co-extruded with PVP, an FDA approved blood expansion product that renders the fibers hydrophilic but inhibits cell attachment. PBS is much more effective at removing PVP than cell culture medium.

Initiate the culture with a small volume of medium in the reservoir bottle (125 mL or less). The volume of medium should be proportional to the number of cells in the cartridge. If the volume of medium in the reservoir bottle is too high the cells will appear to go into a “stasis”, or lag phase due to a low cell density. Increase the medium volume as the cell numbers increase**. Lag phase is characterized by the presence of viable cells but no glucose consumption.**

Always use a pipette to transfer medium - don’t pour from the medium bottle.

Glucose depletion in your media is one of the limiting factors to healthy logarithmic cell growth. When the glucose levels have dropped to less than 50% of its original concentrations it is time to change the medium.

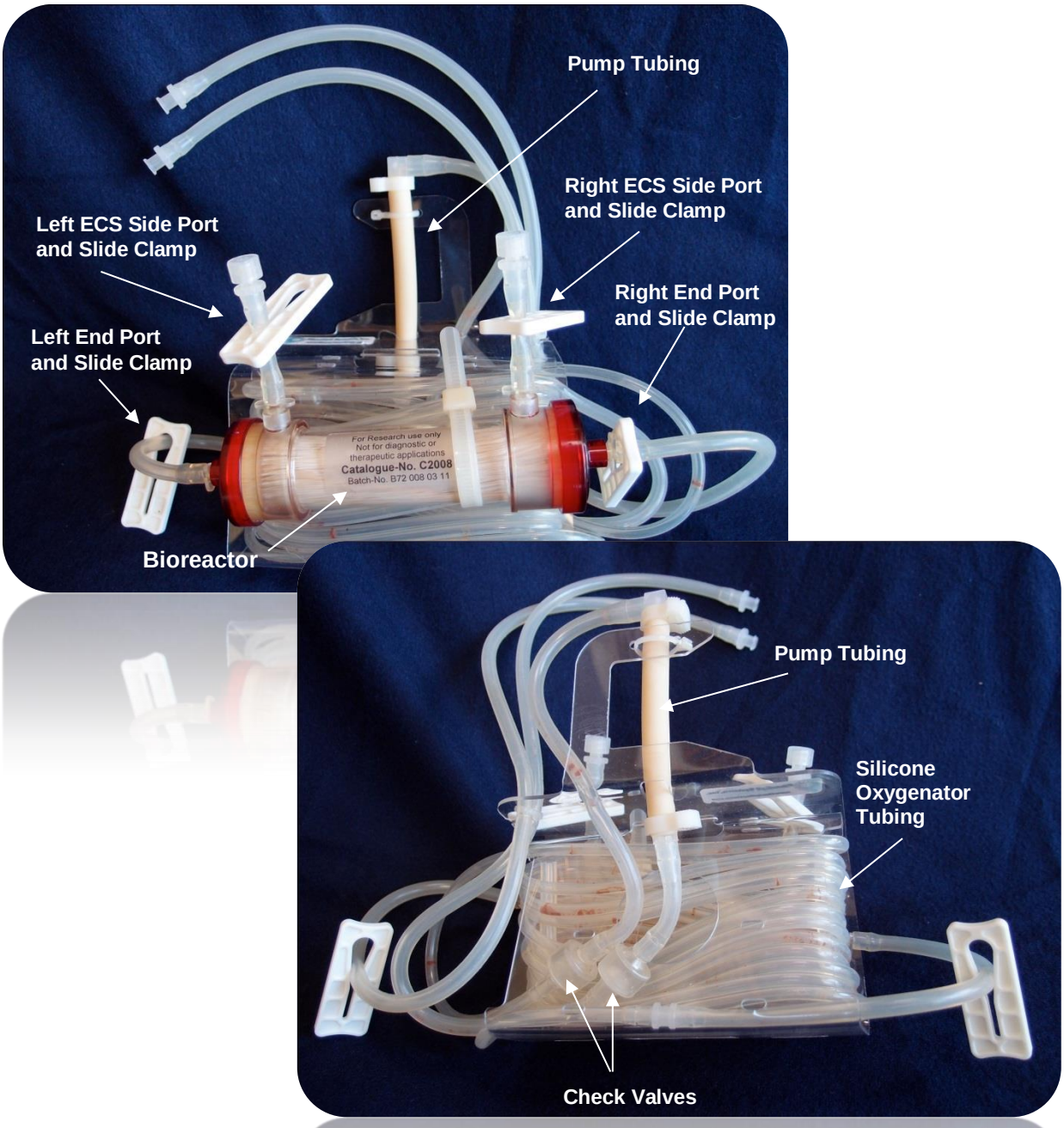
The use of CDM-HD or a serum-free media is highly recommended since it is supplemented with glucose and HEPES. It is suggested that serum, if required, be dropped to as low as 2% to slow down cell growth and therefore the utilization of glucose. This media adaptation should be accomplished

once cells are inoculated in the hollow fiber system and have achieved high cell density, usually after one week. With CDM-HD no adaptation is required.

Follow the step-by-step directions below and using the labeled diagrams of the hollow fiber as your guide equilibrate and pre-culture your system. The FiberCell® Systems Video Manual provides step by step video instructions on how to perform these steps as well.

System Components

Module and flowpath assembly



Reservoir Bottle and Cap Assembly Sterilization

Each cartridge comes with two short pieces of tubing in the outer bag. Being careful not to tear the inner bag, remove the two pieces of tubing. These will be connected to the cap before autoclaving.

Figure 5. Reservoir Cap Assembly and Reservoir Bottle.

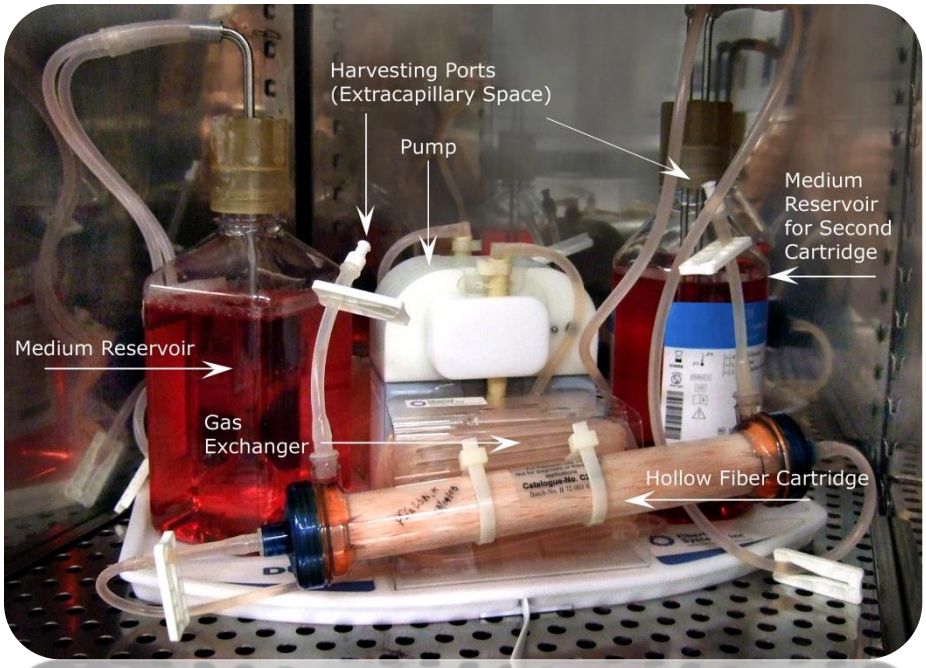
1. Hold the reservoir cap up to the bottle intended to use for pre-culture. The stainless steel tubes should reach within an inch of the bottom of the bottle in order to maintain a constant media flow through the cartridge. If the tubes are too high, wet the tubing around the cap with DI water and they will slide up and down easily. Adjust to correct height. If using the 45 mm cap, the tubing is not adjustable.
2. Attach the two pieces reservoir cap tubing supplied with each cartridge to the hose barb fittings on the reservoir cap and cover with aluminum foil. Cover lower end of tubing on reservoir cap with aluminum foil and secure with autoclave tape.
3. Place the reservoir cap assembly into an autoclave bag.
4. Autoclave the reservoir cap at 120 –130 °C for 45- 60 min.
5. If your autoclave does not have a dry cycle, place the autoclave bag into the laminar flow hood immediately after removal from the autoclave. *The wet paper side of an autoclave bag is not a barrier to contamination.*

After autoclaving, in laminar flow hood, using sterile technique, (refer to video CD), perform the following steps:

1. Take the reservoir cap out of the autoclave bag and place into a sterile bottle or directly onto a sterile bottle of PBS.
2. Remove the FiberCell® Systems module (with flow path) from the sterile package. All of the open ends are sealed with luer caps.
3. Connect the module inlet and outlet tubing to the two luer fittings on the reservoir bottle cap

NOTE: Use a pipette to transfer medium - don't pour from the medium bottle.

- Check that the inlet and outlet slide clamps are in the OPEN position.
- Perfuse medium through the flowpath circuit by pumping the compression tubing. Keep going until the entire circuit is filled.
- Tilt the cartridge with the outlet side up to purge any air bubbles that may have collected in the fibers or at the inlet and outlet ends of the bioreactor.



Pre-culture

You are now ready to pre-culture the cartridge in preparation of cell inoculation.

Materials

Cells will not be inoculated until several days later. Please have the following materials on hand in the hood prior to starting:

- ✓ Sterile PBS
- ✓ FiberCell® Systems culture module
- ✓ FiberCell® Systems reservoir cap, autoclaved, with tubing attached
- ✓ 20 cc sterile syringes (luer-lock) (60 mL for larger cartridges)
- ✓ Alcohol pads
- ✓ Spray bottle containing 70% ethanol
- ✓ Large bore needles
- ✓ 50 mL conical centrifuge tubes
- ✓ 25 mL or 50 mL pipettes
- ✓ Sterile 250 mL plastic Nalgene bottle (38 mm cap) or sterile 250 mL glass bottle with black phenolic cap (33 mm)
- ✓ If you are using a 500 mL bottle of Gibco PBS you will require the 45 mm reservoir cap

The system must be pre-cultured in the incubator for at least 24 hours (we recommend 72 hours) with 500 mL of PBS followed by three changes of cell culture medium. The purpose of this pre-culture is to:

- remove the wetting agent from the fibers
- equilibrate the system with growth medium and serum proteins
- verify that the system is leak free
- perform a sterility check

Prime and fill the cartridge with PBS

1. Check that the left and right end port slide clamps are in the OPEN position and both left and right ECS ports are closed.
2. Perfuse medium through the flowpath circuit by pumping the compression tubing with your fingers until the circuit is filled and no bubbles come from the stainless steel tubing inside the reservoir bottle.
3. Tilt the cartridge with the right side up to purge any air bubbles that may have collected in the fibers or at the ends of the bioreactor.

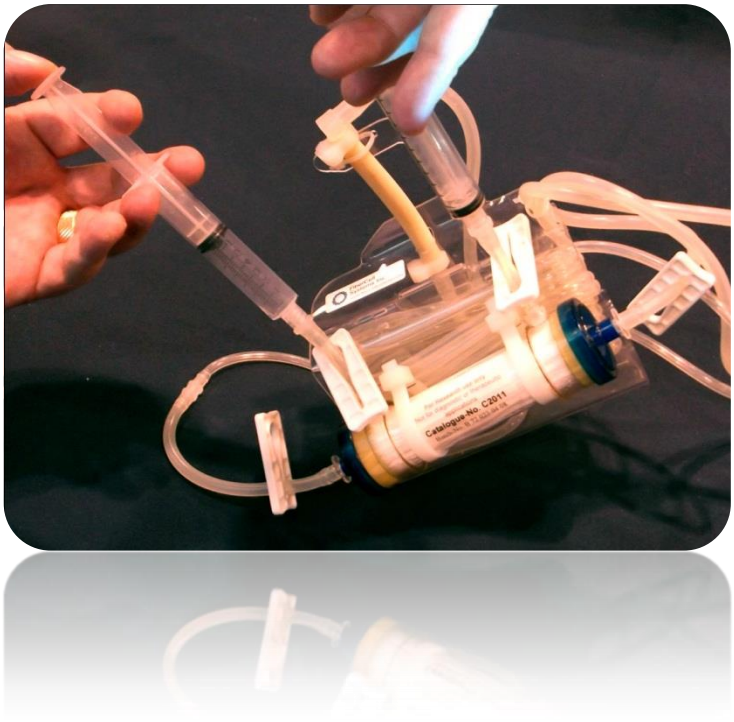
Fill the ECS with PBS

1. Close the left and right end port slide clamps on the cartridge to isolate the bioreactor from the flow path.
2. Attach a sterile syringe (20 – 60 mL depending on the bioreactor size) to one ECS side port.
3. Add ~50 mL of PBS to a 50 mL conical centrifuge tube.
4. Fill a second syringe with PBS using a large gauge needle and connect it to the other ECS side port.
5. Open the left and right ECS slide clamps.
6. Inject the PBS into the ECS displacing the air into the other syringe. If the ECS is not completely filled with medium, repeat, dislodging all air bubbles.
7. Close the ECS port clamps, remove the air from the syringes and use the syringes as caps. Remember to use fresh syringes for any subsequent manipulations.
8. Open the left and right end port slide clamps
9. Place the cartridge onto the Duet and run PBS through the system at a flow rate of 15 - 20 for a minimum of 24 hours. At this point the cartridge may be run with the PBS for several weeks if necessary.

Remember to use the alcohol swabs to clean up any medium on the luer fittings or cartridge. Whenever the ECS side ports do not have a syringe or cap on be sure to have the slide clamps closed, this will prevent any excess medium from collecting on the fittings or leaking.

In the event of ECS drainage, (ECS fills with air overnight during pre-culture) raise the level of the reservoir bottle so that the level of the medium in the reservoir is higher than the ECS. This will generate sufficient hydrostatic pressure to keep the ECS filled with medium. Also, ensure that the ECS port slide clamps are closed and the luer caps or syringes are tightly fitted.

After this 24 hour-flush, there will be two more changes of medium in the system. One basal medium without serum or growth factors (or serum free medium) and then a second change to complete medium containing serum, antibiotics and any other additives. Finally, perform a fresh change of medium for cell inoculation and initial culture. When the cells are established, i.e., consuming one gram of glucose a day or more, adaption to serum free media or CDM-HD can be performed. CDM-HD requires little or no adaptation. Simply replace the fetal bovine serum in the DMEM with 10% CDM-HD. CDM-HD instructions may be found at: www.fibercellsystems.com/CDM-HD



First Media Change

1. This first liquid change will be using classical media/serum free media. Replace the PBS with a 500 mL bottle of classical media.
2. Close the left and right end port slide clamps. Close the left and right ECS port slide clamps.
3. Change the medium in the ECS by filling a 20 mL syringe with the new medium and attaching it to the left ECS side port. Place an empty syringe onto the right ECS side port.
4. Open the ECS port slide clamps.
5. Tilt the cartridge up on the right side and exchange the medium in the ECS by slowly pushing the new medium in from the left syringe and floating the old medium out the right ECS port.
6. Remove the PBS from the right syringe and reattach it to the ECS port.
7. Close the clamps on the left and right ECS ports. **Remember to open the left and right end port slide clamps!**
8. Let the media circulate for a minimum of 24 hours, placing the system back in the incubator.

Second Media Change

1. Follow steps in first media change.
2. If using a basal medium, change out the DMEM for DMEM plus 10% fetal bovine serum and any other additives. Allow this to circulate for 24 hours.

Final Media Change

Put on a fresh change of DMEM plus 10% fetal bovine serum, 125 mL. Larger volumes have been used as a convenience to this point however during the initial seeding period it is important that the volume be no more than 125 mL. The volume of media in the reservoir bottle needs to remain proportional to the number of cells in the cartridge.

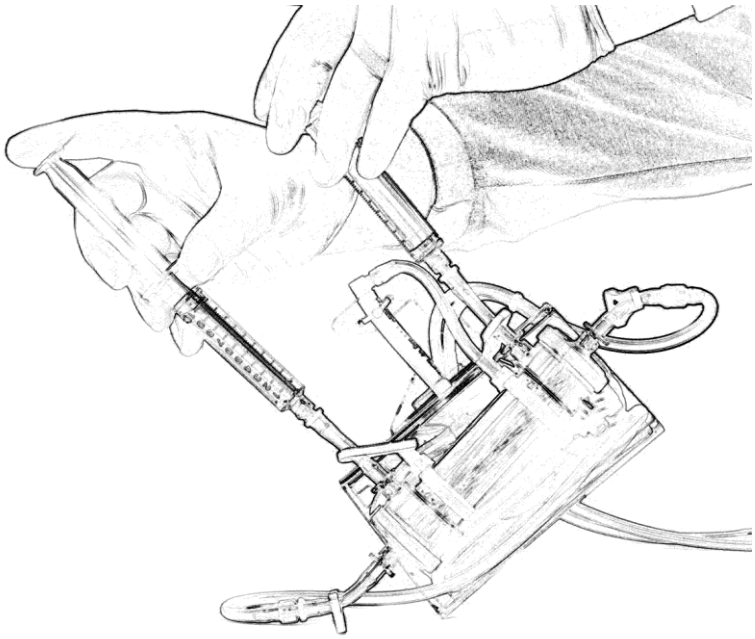


Figure 8. Tilting the cartridge upwards when filling the ECS or harvesting facilitates the removal of air bubbles

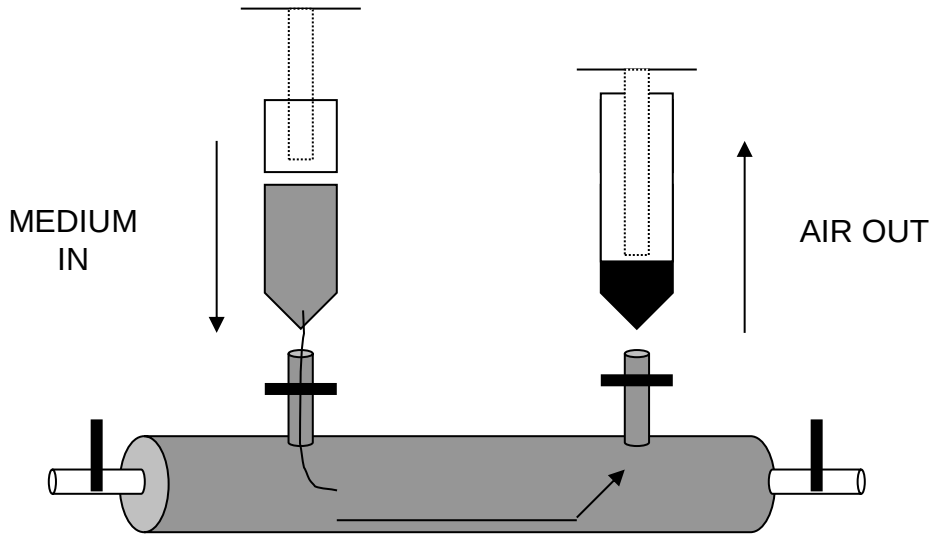


Figure 9. Schematic of filling the ECS. The left and right endport slide clamps should be closed, the left and right ECS sideport slide clamps should be open.

You are now ready to inoculate with cells. Please refer to either the hybridoma or recombinant protein sections below.

Inoculation with Cells

Make sure that the cells are at least 90% viable. Minimize the amount of time between cell harvesting and inoculation. Re-suspend the cells in the same conditioned medium that they have been growing in as this will contain useful growth factors that should not be discarded. Recommended cell numbers are given below:

- Hybridomas and suspension cells: a minimum of 10^8 total cells.

Please refer to the FiberCell® Systems Video CD Instruction Manual for visual instructions.

Procedure

1. Close the left and right side ports of the cartridge.
2. Remove both ECS port syringes.
3. Replace one with a fresh syringe.
4. Fill a second syringe with 20 mL of cell culture suspension.
5. Attach syringe containing cells to the other ECS port.
6. Displace the media containing cells into the empty syringe. Push gently to avoid creating bubbles or foam.
7. Gently flush the cell suspension back and forth 3 - 4 times through the ECS to uniformly distribute cells throughout the fiber bundle. Allow ½ of the cell suspension to remain in each syringe.
8. Crack the reservoir cap by ½ turn. Close one ECS port slide clamp and gently push the suspension in the other syringe through the fibers and into the reservoir bottle. The cells will remain in the cartridge while the excess medium will go into the reservoir bottle.
9. Close the ECS slide clamp and repeat with the opposite syringe, remembering to close ECS ports after expelling cell suspension.
10. Tighten the reservoir cap.
11. Allow the cartridge to sit in the hood for one hour, rotating it 180 degrees after 30 minutes.
12. Leave these syringes on to help guard against contamination.
13. Place the cartridge onto the pump and set pump speed to 20 - 25.

Change to a 250 mL bottle of complete medium when the glucose has been depleted by half. This will generally be when the glucose level has reached 2 grams per liter or less. You can purchase an inexpensive glucometer (the kind that diabetics use) at most any drug store. We recommend the Roche Accu-check Cat# 03149137001, or equivalent chemistry.

Once the glucose level has been depleted by half, change to a 500 mL bottle of complete media.

Once the glucose has been depleted by half, change to 1L if desired. Once the glucose consumption rate is 1.0 grams per day or higher, harvesting may begin.

FiberCell Hybridoma Cartridge Specifications

CATALOG NUMBER	MWCO (@50%)	AREA cm^2	FIBER TYPE	ECS VOL (ml)	GFR#	INOCULATE##
C2011, C5011	20 kDa	3000 cm^2	Polysulfone	20	130	1 X 10^8

Catalog numbers C2011 and C5011 are recommended for hybridoma cell culture.

FOOTNOTES: GFR#units: mm Hg/cm^2/min

INOCULATE## minimum number of cells

Protocol Summary – Routine Daily Maintenance

DAY	CHECK GLUCOSE	CALCULATE RATE	RESERVOIR VOL.	ACTION (S)
0	YES	N/A	125mL	• INOCULATE CELLS • ATTACHMENT • FLOW RATE AT 20 ml/min
1	YES	YES	125mL	• DOUBLE MEDIUM IN RB IF GLUCOSE IS 50% DEPLETED
2 - 5	YES DAILY	YES DAILY	200mL – 500mL	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED • INCREASE FLOW RATE TO 50 ml/min
5 - 7	YES DAILY	YES DAILY	500mL – 1000mL	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED • INCREASE FLOW RATE TO MAXIMUM • HARVEST SECRETED PROTEIN FROM ECS
7 - 10	YES DAILY	YES DAILY	1000mL (2 L to 4 L for large cartridges)	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED • HARVEST Mab FROM ECS EVERY TWO DAYS
10 +	YES DAILY	YES DAILY	1000mL (2 L to 4 L for large cartridges)	• • REPLACE MEDIUM BOTTLE WHEN GLUCOSE IS 50% DEPLETED HARVEST MAB FROM ECS EVERY TWO DAYS • REDUCE SERUM IF DESIRED • MONITOR CELL PACKING IN ECS AND FLUSH AS REQUIRED

Measuring Glucose in Reservoir Bottle

Unlike static dish or flask culture where one can inspect the morphology of the cells by microscope, hollow fiber culture requires an alternate, and much more quantitative method to determine the health of the cells. FiberCell® Systems PS fibers are white in color making it nearly impossible to observe the high numbers of cells inside the cartridge. Measurement of glucose and determination of glucose rates should be considered mandatory procedures for successful hollow fiber cell culture.

The **rate** of glucose consumption is directly related to growth rate, cell number and metabolic activity of the cells in the cartridge and is your window into the metabolic state of the cells. It is also a signal provided by the cell in response to perturbations in culture protocols, e.g., medium change, serum reduction, glucose depletion, lactate accumulation or viral infection. The rate of glucose consumption can be used to determine the number of cells to be removed during each harvest. For the medium sized cartridges, C2011 and C2008, the glucose rate should ideally be between 1.0 and 1.5 grams per day. For the C5011 the ideal glucose uptake rate is between 2.0 and 2.5 grams per day. For the C2018 and C2003 cartridges the ideal glucose uptake rate is between 2.5 and 4.0 grams per day. The **total amount** of glucose present in the reservoir bottle is an indication of when to change the medium to a fresh bottle of medium. A general guide is that the medium should be replaced when the glucose has been 50% depleted. You may find that certain cell types can tolerate much lower levels of glucose without suffering total cell death. This can be exploited to leave a culture unattended for a period as long as a two-day weekend.

We recommend the Roche Accu-check Cat#03149137001, or equivalent chemistry.

Experience with your cell line helps, but it will take some experimentation to find the optimum conditions for growth.

Remembering that the rule of thumb is to change the media when the starting glucose is 50% depleted, chart the consumption rate of glucose by taking samples frequently the first day, using your monitor.

In the example below, your glucose monitoring indicates a media exchange within about 12 hours. By 20 hours, when the depletion rate has decreased, your cells are no longer in logarithmic growth and have become stressed. It is advisable to exchange the media no later than the 16th hour.

Example:
Starting glucose..... 0.45 mg/mL
after 8 hours..... 0.35 mg/mL
after 12 hours..... 0.27 mg/mL
after 16 hours..... 0.20 mg/mL
after 20 hours..... 0.18 mg/mL

The goal is to adjust glucose levels so the required media exchange is convenient with your laboratory schedule, i.e., every 24 or 48 hours.

Adjusting media volume or cell concentration can achieve this correction.

Harvesting Antibody

There are two methods for harvesting antibody from the cartridge and the method used depends upon the glucose uptake rate. For glucose rates of 1 gram per day or less only the low glucose rate harvest should be used. When the glucose uptake rate is above 1 gram per day cells need to be removed from the cartridge. In this case the low glucose rate harvest should be performed first, followed by the high glucose rate harvest.

Low Glucose Rate Harvest

If the glucose rate is below 1000 mg per day, harvesting should be done so that only a few cells are removed.

Equipment and Materials

- ✓ FiberCell® Systems cartridge
- ✓ 20cc sterile syringes (luer-lock) (60 cc for larger cartridges)
- ✓ Alcohol pads
- ✓ Spray bottle containing 70% ethanol or isopropyl alcohol
- ✓ Cell culture media of choice

Procedure

1. Close the left and right end port slide clamps. Make sure the ECS port slide clamps are closed.
2. Fill a 20 mL syringe with the fresh complete medium. Remove the syringe off the left ECS port and replace with the syringe containing fresh medium. The right ECS port syringe should be empty and ready to go. This syringe may be used to collect the supernatant or a fresh syringe may be used. Open both the left and right ECS port slide clamps.
3. Tilt the cartridge up on the right side and exchange the medium in the ECS by slowly pushing the new medium in from the left syringe and floating the supernatant out the right ECS port syringe. (Please refer to picture next to first media change). This will represent your low glucose rate harvest.
4. Close both the ECS slide port clamps. Remove the syringe containing the harvest and replace with fresh sterile syringe.
5. Before putting back into the incubator, be sure that both the inlet and outlet end port slide clamps are open. Also be sure that the ECS slide port clamps are closed.

High Glucose Rate Harvest

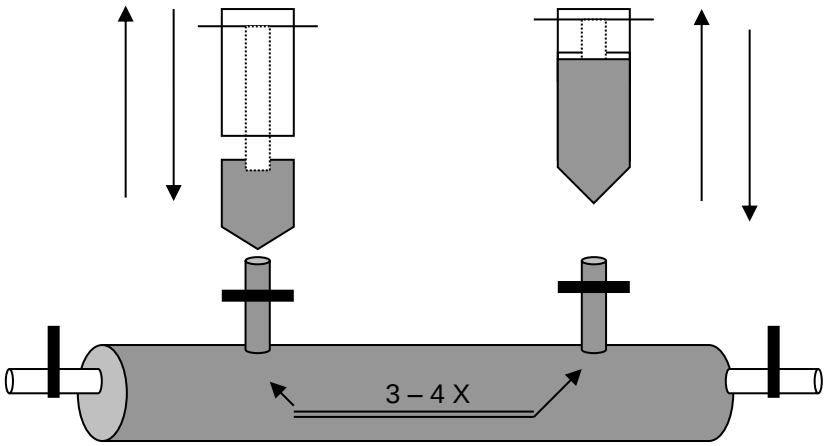
Use this procedure when the glucose rate is 1000 mg per day or above. The cell mass needs to be controlled and cell numbers reduced. The cell pellet may be 1 - 4 mL of packed cells. The high glucose rate harvest generally follows the low glucose rate harvest.

Equipment and Materials

- ✓ FiberCell® Systems cartridge
- ✓ 20 cc sterile syringes (luer-lock) (60 cc for larger cartridges)
- ✓ Alcohol pads
- ✓ Spray bottle containing 70% ethanol or isopropyl alcohol

Procedure

1. Close the left end port slide clamp (right end port remains open).
2. Attach two fresh 20 mL syringes to the ECS ports.
3. Crack the reservoir bottle cap about 1/4 turn
4. Open the right ECS port slide clamp
5. Pull 10 mL into the right syringe (you are pulling medium out of the reservoir bottle, through the fibers, into the syringe
6. Close the right ECS port slide clamp
7. Open the left ECS port slide clamp
8. Pull 10 mL of medium into the syringe
9. Close the right end port slide clamp
10. Open both ECS port slide clamps
11. Swish the medium between the two syringes, 2 - 3 times, gently (if a marked decrease in cell mass is desired more vigorous swishing is necessary.
12. Push all the media into one of the syringes, doesn't matter which one.
13. Close the ECS port slide clamps. Remove the syringe containing the medium and empty it into a 50 mL conical.
14. Replace the syringe
15. Open the left and right end port slide clamps.



- Transfer the cell wash to the outlet side syringe.
- Close off the ECS sideport slide clamps.
- Remove the syringe and transfer the cell to a 50 cc conical centrifuge tube.
- Replace the syringes and remove all traces of medium from the sideport ECS luer fittings with an alcohol swab.

- **Open the inlet and outlet slide clamps – DON'T FORGET, 60 MIN WITHOUT MEDIUM FLOW WILL KILL THE CELLS.**

- Place the module back in the incubator making certain that:
    - The inlet and outlet slide clamps are open
    - The flowpath tubing is free from kinks
    - The perfusion pump is on.

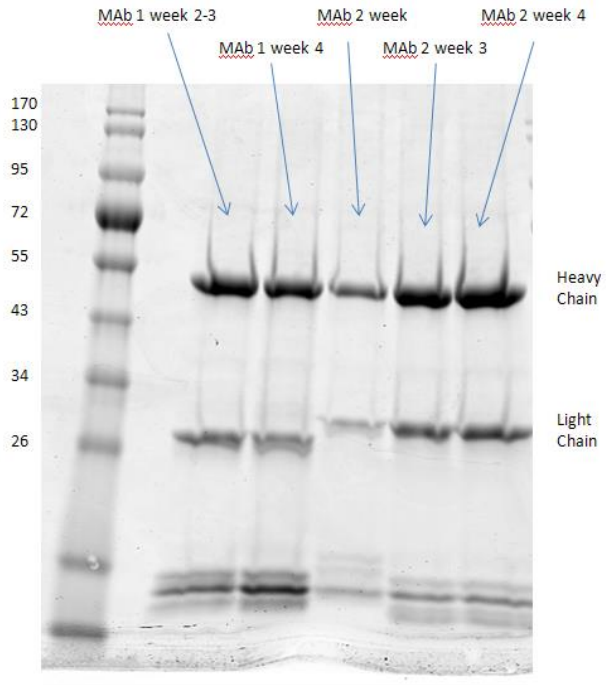
CDM-HD and Serum Reduction

- Most hybridoma cell lines should be started with 10% serum in the medium. If the cells are already adapted to reduced serum use medium at that serum concentration and 50% conditioned medium at inoculation.
- After the cells have reached 500mg glucose/day the serum can be reduced from 10% to 7.5%. Monitor the glucose rate. It may take several days for the cells to adapt to the reduced serum, which will be reflected by a recovery of the glucose rate.
- Keep the serum at 7.5% at the next medium change
- At the next change of medium, reduce the serum to 5% for the next two medium changes. Monitor the glucose rate. If the rate doesn't recover allow the cells more time to adapt.
- After this point reduce the serum by 1% at each feeding. Monitor the glucose as before. If the rate doesn't recover allow the cells more time to adapt.
- Most cell lines will be able to adapt to 1 – 3% serum.
- The same serum concentration should be used in the ECS as in the reservoir bottle.

Medium Volume in Reservoir Bottle

- The volume of medium in the reservoir bottle should be proportional to the number of cells inside the cartridge. If the volume is too great it will have the same effect as under-inoculating the cartridge i.e. the cells will appear to go into stasis.
- When cells are first inoculated the volume of medium in the reservoir bottle should be 125 mL and increase along with the cell number in the cartridge.
- The medium sized cartridge (C2011) should have as much as a liter of medium in the reservoir bottle, the C5011 should have as much as 2 liters of medium in the reservoir. This can be accomplished by attaching 2 medium bottles in series using two reservoir caps to the cartridge.
- Some hybridoma cell lines will not tolerate a volume of more than 500mls in the reservoir bottle. If the glucose rate drops when the volume is increased to 1 liter from 500 mL return to a 500 mL reservoir bottle.

- Most hybridoma cell lines should be started with 10% serum in the medium. If the cells are already adapted to serum free medium use the serum free medium supplemented with 2 – 5% serum concentration and 50% conditioned serum free medium at inoculation. Serum also contains attachment factors that are important for cell adherence. If it is a requirement that the cells are not exposed to serum this step can be omitted. Good results have been obtained when using CDM-HD and many different types of hybridomas.
- CDM-HD does not require any adaptation. The cartridge can simply be switched to medium containing CDM-HD rather than serum when the glucose rate reaches 1 gram per day.
- After the cells have reached ~ 250 mg/glucose/day the serum can be reduced 50%. Monitor the glucose uptake rate. It may take several days for the cells to adapt to the reduced serum, which will be reflected by a recovery of the lactate rate.
- Keep the serum at that level during the next medium change
- At the next change of medium, reduce the serum another 50% for the next two medium changes. Monitor the glucose rate. If the rate doesn't recover allow the cells more time to adapt.
- After this point eliminate serum addition and switch to 100% serum free medium. Monitor the glucose as before. If the rate doesn't recover allow the cells more time to adapt.



Unpurified supernatant of monoclonal antibody from C2011 cartridge using CDM-HD. Note that the antibody is very clean and free from contaminating proteins. Data courtesy of: Dr. Erin Bromage c/o the US Veterinary Immune Reagents Network.

- Most hybridoma cell lines will be able to adapt to serum free medium growth in FiberCell® Systems hollow fiber bioreactors.
- Cholesterol dependant cell lines such as human heterohybridomas and NSO lines will have difficulty adapting to completely serum free conditions. Synthetic cholesterol will bind to the silicone tubing. In these cases CDM-HD with .5-1% serum has been used successfully.

Recombinant Protein Production

Recombinant proteins can be produced from any stably transfected mammalian cell line. There is also a protocol for production of proteins from stably transfected S2 cell lines. Make sure that the cells are at least 90% viable. Minimize the amount of time between cell harvesting and inoculation. Re-suspend the cells in the same conditioned medium that they have been growing in as this will contain useful growth factors that should not be discarded. Recommended cell numbers are given below:

- Adherent Cells such as CHO and HEK 293: use the equivalent of 50% confluence of the fiber surface area. This will be 6 - 8 T-175 flasks or the equivalent. Otherwise plan on seeding 1 X 10^8 cells for the medium sized cartridges and 2 - 4 X 10^8 cells for the larger cartridges.

Inoculation

Please refer to the FiberCell® Systems Video CD Instruction Manual for visual instructions.

When producing a protein of 120 kd or larger the C2011 or C2018 20 kd MWCO cartridge should be used. This fiber allows free exchange of medium across the fiber and excess volumes can be ultrafiltered into the reservoir bottle. **When inoculating the C2011, C5011 and C2018 cartridges the same protocol for inoculating hybridomas should be used**. When producing proteins between 20 kd and 120 kd the 5 kd MWCO fiber should be used (C2008, C2003). The low molecular weight cut off does permit the exchange of significant volumes of medium so the protocol below should be followed.

Procedure for 5 kd MWCO cell inoculation:

1. Close end port slide clamps
2. Attach fresh syringes on ECS side ports, empty 20 mL syringe with plunger pulled back so full of air.
3. Open ECS side port slide clamps
4. Tilt cartridge and withdraw medium from ECS to empty syringe pulling air from other syringe (this keeps you from just pulling air from the hood into the ECS)
5. Note volume removed, should be around 10 - 12 mL
6. Resuspend cells to be inoculated in the volume removed plus 2 mL
7. Fill syringe with cell inoculum
8. Attach filled syringe to ECS side port. You should now have an empty syringe on right and syringe with cells on left
9. Depress plunger and push in cells, swish back and forth, 1 mL remains in each syringe
10. Close one ECS side port clamp, push in one mL of excess medium and repeat with the other ECS port. It should be possible to easily get 2 mL through the fiber.

Change to a 250 mL bottle of complete medium when the glucose has been depleted by half. This will generally be when the glucose level has reached 2 grams per liter or less. You can purchase an inexpensive glucometer (the kind that diabetics use) at most any drug store. We recommend the Roche Accucheck Cat # 03149137001, or equivalent chemistry.

Once the glucose level has been depleted by half, change to a 500 mL bottle of complete media.

Once the glucose has been depleted by have, change to 1L if desired. Once the glucose consumption rate is 1.0 grams per day or higher, harvesting may begin. With the 20 kd MWCO cartridges the harvesting protocol is the same as for the hybridoma protocol. For the larger cartridges, C2018 and C2003 simply use a 60 mL syringe rather than a 20 mL syringe. For the 5 kd MWCO cartridges follow the high glucose rate harvest protocol below.

Harvesting

If the glucose rate is below 1000 mg per day, harvesting should be done so that only a few cells are removed. When harvesting from the 20 kd MWCO cartridges the harvesting protocols for both low and high glucose rates are the same as for the hybridoma protocol. As a general rule harvesting should be performed every day as opposed to every other day as for hybridomas.

Procedure for 5 kd MWCO High Glucose Rate Harvest

It is difficult to ultrafilter more than 1 - 2 mL of medium through the 5 kd MWCO fiber. For the high glucose rate harvest it is still useful to pull a small amount of medium through the fibers, but filling a syringe with 10mls of medium will provide sufficient volume to flush cells from the cartridge

1. Close left end port slide clamp, leave right end port slide clamp open.
2. Attach fresh syringes on ECS side ports, one filled with 10 mL of medium, the other empty with the plunger all the way down.
3. Open the left ECS side port slide clamp
4. Open the reservoir bottle cap ¼ turn
5. Pull one mL of medium into the left syringe.
6. Close the left ECS side port clamp, open the right ECS side port
7. Pull 1 mL of medium into left syringe
8. Close right end port slide clamp, open left ECS side port slide clamp
9. Swish medium back and forth between the syringes 3-4 times. Push all the medium into one syringe. This is your harvest
10. Close left and right ECS side port slide clamps
11. Open left and right end port slide clamps
12. Place harvest into 50 mL conical tube and spin down
13. Re-attach syringes to ECS side ports, these will serve as end caps.

Adherent and Non-adherent Cells

Most cell types will adhere to the fibers to some degree with the exception of true suspension cells such as lymphocytes. Hybridoma cell lines will attach to the fiber over a period of a week or so. Some cell types require immediate adherence. These include HEK 293, CHO and HELA cells. Generally these procedures will generate a mixed population of cells in suspension and cells bound to the fiber. For cells that require immediate attachment to grow properly it is important to pre-culture the cartridge with PBS first. Cell inoculation numbers will be different for suspension (cells that do not require immediate attachment) cells versus adherent cells. For adherent cells an inoculation number that is equal to 50% of the surface area of the fibers should be used. For suspension cells 10^8 cells (C2011, C2008) to 4 X 10^8 cells (C2018, C2003) should be used.

Daily Maintenance and Feeding

Please refer to specific sections of this manual for particular cell types. Following are some general guidelines for daily maintenance and feeding.

- Initiate the culture with a small volume of medium in the reservoir bottle (125 mL or less) The volume of medium should proportional to the number of cells in the cartridge. If the volume of medium in the reservoir bottle is too high the cells will appear to go into a "stasis". They remain viable but do not divide or consume glucose. If this occurs reduce the volume of medium in the reservoir bottle or inoculate additional cells.
- Hybridoma cultures should be harvested every other day.
- Recombinant protein cultures should be harvested every day.
- Harvest method is based upon the lactate or glucose rate. If the rate is higher than 1,000mgs/day (4,000 mg/day for the large cartridges) the cell population should be reduced by using the high glucose rate harvest.
- Medium should be changed based on the total amount of glucose in the medium. A general rule of thumb is to change the medium when 50% of the glucose is consumed.
- Do not allow the cartridge to become over-grown with cells. This is based upon the glucose rate and the occurrence of anaerobic metabolism.
- The degree of anaerobic metabolism can be determined by calculating the glucose consumption/lactate production ratio. For every mole of glucose consumed, one mole of lactate should be produced under perfectly aerobic cell culture conditions. This ratio shifts towards two moles of lactate produced for each mole of glucose consumed as the amount of anaerobic metabolism increases. If this occurs remove cells until the ratio is near one again.
- Cells are less tolerant of high levels of lactate or lower glucose concentrations at lower cell densities i.e. when the culture module is early in its growth phase.
- Lymphocytes are very sensitive to lactate and may die if the lactate concentration exceeds 700 – 900 mg/L.

Measuring Glucose in the Reservoir Bottle.

The same protocol used for monitoring glucose for hybridoma culture should be used for recombinant protein production culture. A 1 mL sample is removed from the reservoir bottle and

assayed for glucose using the glucose meter. The total amount of glucose indicates when it is time to change the medium, the rate of glucose consumption determines the type of harvest to be performed. When over 1 gram per day the high glucose rate harvest should be performed, when it is below 1 gram per day the low glucose rate harvest should be performed. The primary difference between the hybridoma protocol and recombinant protein protocol is that during recombinant protein production the harvests should be performed every day, if possible. The harvest protocols are slightly different for the 5 kd MWCO fiber as it is not easy to ultrafilter large volumes of medium across the fiber.

Cartridge Selection

- The mode of sequestration of secreted proteins in hollow fiber bioreactors will either be the small volume/high concentration ECS harvesting or the large volume/low concentration/Reservoir Bottle alternative. It is always desirable to retain the proteins in the ECS unless they possess some sort of inhibitory activity.
- Secreted and recombinant protein may range in size from small peptides to protein molecules larger than 150 – 200 kDa.
- The selection of pore size may sometimes be a compromise between the desired small volume/high concentration ECS harvesting and large volume/low concentration/Reservoir Bottle collection.
- Please refer to the following Table for a summary of the filtration characteristics of FiberCell® Systems hollow fiber cartridges**.**

FiberCell® Systems Recombinant Protein Cartridge Specifications

CATALOG NUMBER	MWCO (@50%)	AREA cm^2	FIBER TYPE	ECS VOL (ml)	GFR#	INOCULATE## (X 10^8 cells)
C2011	20 kDa	3000 cm^2	Polysulfone	20	130	Inoculate cell number equal to 50%
C2008	5 kDa	3000 cm^2	Polysulfone	20	12	confluence of fiber surface area
C5011	20 kDa	3000 cm^2	Polysulfone	20	130	Inoculate cell number equal to 50%
C2018	20 kDa	1.2 m^2	Polysulfone	70	130	Same as above
C2003	5 kDa	1.2 m^2	Polysulfone	70	12	Same as above

FOOTNOTES:
GFR#units: mm Hg/cm^2/min
INOCULATE## or a number of cells that will provide ~50% fiber surface confluence.

Cartridge Selection Guide

Anchorage dependent cell lines require a pre-culture of 2 changes of sterile PBS prior to pre-culture with cell culture medium. Inoculation with 10% FBS is recommended. If the 5 kd fiber is used the ECS should be flushed with at least 3 changes of PBS prior to initiating pre-culture with cell culture medium.

C2011, C2018 High molecular weight cut-off fiber for trapping larger molecules**.** The high gross filtration rate of this fiber is desirable for higher cell growth rates. It will usefully trap secreted proteins that are larger than 100 kd in size.

C2008, C2003 Low molecular weight cut-off fiber for trapping smaller molecules. This fiber will usefully trap proteins in the range of 20 kd up to 100 kd. For proteins larger than 100 kd C2011 or C2018 should be used.

Medium Volume in Reservoir Bottle

- The volume of medium in the reservoir bottle should be proportional to the number of cells inside the cartridge. If the volume is too great it will have the same effect as under-inoculating the cartridge i.e. the cells will appear to go into stasis.
- When cells are first inoculated the volume of medium in the reservoir bottle should be small and increase along with the cell number in the cartridge.
- After 7-10 days of culture the cartridge should approach maximum cell numbers. The medium sized cartridge (C2011) should have as much as a liter of medium in the reservoir bottle, the larger cartridge (C2018) should have a minimum of 2 liters of medium and as much as 4 liters of medium in the reservoir. This can be accomplished by attaching multiple medium bottles in series to the cartridge.

Protocol Summary – Routine Daily Maintenance

DAY	CHECK GLUCOSE	CALCULATE RATE	RESERVOIR VOL.	ACTION(S)
0	YES	N/A	100	• INOCULATE CELLS • ATTACHMENT • FLOW RATE AT 20 ml/min
1	YES	YES	100	• DOUBLE MEDIUM IN RB IF GLUCOSE IS 50% DEPLETED
2 - 5	YES DAILY	YES DAILY	200	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED INCREASE FLOW RATE TO 50 ml/min
5 - 7	YES DAILY	YES DAILY	500 – 1000	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED INCREASE FLOW RATE TO MAX • HARVEST SECRETED PROTEIN FROM ECS

7 - 10	YES DAILY	YES DAILY	1000 (2 L to 4 L for large cartridges)	• DOUBLE MEDIUM VOL IN RB IF GLUCOSE IS 50% DEPLETED • HARVEST SECRETED PROTEIN FROM ECS DAILY
10 +	YES DAILY	YES DAILY	1000 (2 L to 4 L for large cartridges)	• REPLACE MEDIUM BOTTLE WHEN GLUCOSE IS 50% DEPLETED • HARVEST SECRETED PROTEIN FROM ECS EVERY DAY • REDUCE SERUM IF DESIRED • MONITOR CELL PACKING IN ECS AND FLUSH AS REQUIRED

CDM-HD and Serum Reduction

- Most secreted protein cell lines should be started with 10% serum in the medium. If the cells are already adapted to reduced serum use medium at that serum concentration and 50% conditioned medium at inoculation.
- After the cells have reached ~ 250 mg/glucose/day the serum can be reduced from 10% to 7.5%. Monitor the glucose rate. It may take several days for the cells to adapt to the reduced serum, which will be reflected by a recovery of the glucose rate.
- Keep the serum at 7.5% at the next medium change
- At the next change of medium, reduce the serum to 5% for the next two medium changes. Monitor the glucose rate. If the rate doesn't recover allow the cells more time to adapt.
- After this point reduce the serum by 1% at each feeding. Monitor the lactate as before. If the rate doesn't recover allow the cells more time to adapt.
- Most cell lines will be able to adapt to 2 - 3% serum.

CDM-HD

- Most secreted protein cell lines should be started with 10% serum in the medium. If the cells are already adapted to a specifically formulated serum free medium use the serum free medium supplemented with 1 - 3% serum concentration and 50% conditioned serum free medium at inoculation.
- CDM-HD will provide excellent results with most cell lines and particularly 293 and CHO lines providing a protein free medium optimized for hollow fiber cell culture.
- No adaptation is required when switching to CDM-HD
- After the cells have reached ~ 250 mg/ glucose /day the serum can be reduced 50%. Monitor the glucose rate. It may take several days for the cells to adapt to the reduced serum, which will be reflected by a recovery of the glucose rate.
- Keep the serum at that level during the next medium change
- At the next change of medium, reduce the serum another 50% for the next two medium changes. Monitor the glucose rate. If the rate doesn't recover allow the cells more time to adapt.

- After this point eliminate serum addition. If the rate doesn't recover allow the cells more time to adapt.
- Many secreted protein cell lines will be able to adapt to serum free medium growth in bioreactors.
- Be sure to monitor protein productivity. Some cell lines will grow well in serum free medium but stop producing the protein of interest.
- As a general rule the more complex and expensive a medium is, the less suitable it is for hollow fiber cell culture.

Open the inlet and outlet slide clamps – DON'T FORGET, 60 MIN WITHOUT MEDIUM FLOW WILL KILL THE CELLS.

- Place the module back in the incubator making certain that:
  - The inlet and outlet slide clamps are open
  - The flowpath tubing is free from kinks
  - The perfusion pump is on.

For a more detailed description on operating your FiberCell® Systems Hollow Fiber Bioreactor, please refer to the FiberCell® Systems User's Manual, which can be found at our website: www.fibercellsytems.com.

For further information please visit our web site at www.fibercellsystems.com or contact FiberCell® Systems technical support at (301) 471-1269.

FAQs and Trouble Shooting

6

Medium

What medium is best? How do I adapt to a serum-free medium?

- Usually the medium that the cells have been adapted to grow in works best for the starting medium.
- Hollow fiber culture allows for easy transition to alternate media, reduced serum, or elevated glucose concentrations. CDM-HD is optimized for hollow fiber cell culture and is the ideal medium for most hollow fiber bioreactor applications and requires no adaptation.
- A transition to an alternate medium, serum concentration, etc. should be made after the cells have adapted to the cartridge and the glucose uptake rate is higher than 1 gram per day, usually at day 5 - 7. At that time one can change the medium in the reservoir bottle in the following manner:
  - First medium change: 75% old medium, 25% new medium
  - Second medium change: 50% old medium, 50% new medium

    - Third medium change: 25% old medium, 75% new medium
- Change the medium in the ECS (during harvesting) with the new formulation of medium as well.
- Monitor the glucose rate to ensure that the cells are not adversely affected

Should I use high glucose medium?

- Medium containing 2.5 g/L of glucose, e.g. RMPI do not usually provide the cells with sufficient glucose for growth in hollow fiber bioreactors where cell densities (adjusted to system medium volume) are 50 - 100 fold higher than in conventional dish culture.
- Use of a high glucose medium, e.g., DMEM with 4.5 g/L of glucose provides sufficient glucose for high-density cultures. Monitor the cell viability to determine the maximum safe lactate level.

Serum

What is the optimum serum concentration?

- Most cells should be started with 10% serum in the medium.
- After the cells have reached ~150 – 250 mg/L glucose/day the serum can be reduced to 7.5%. Monitor the glucose rate. It may take several days for the cells to adapt to the reduced serum which will be reflected by a recovery of the glucose rate.
- At the next change of medium, reduce the serum to 5%. Monitor the glucose rate.
- After this point reduce the serum by 1% at each feeding. Monitor the glucose as before.
- Most cell lines will be able to adapt to 2 - 3% serum. Serum levels below this amount may support cell growth but protein secretion may be inhibited. It is not recommended to use serum levels below 2%.

What about serum free medium formulations?

- Cells already adapted to a specially formulated serum free medium will do fine in hollow fiber bioreactors.
- Expensive and complicated serum free mediums generally do not provide a performance advantage over CDM-HD except for cholesterol dependant cell lines.
- Since the autocrine factors secreted by the cells are concentrated within the ECS most cells will exhibit enhanced growth and/or protein secretion levels.
- Cells can be adapted to serum free culture media using the stepwise transition from old to new medium described above. This can be more efficient than attempting to adapt the cells to serum free medium and then loading into the hollow fiber cartridge. Cells growing at the high densities found in a hollow fiber cartridge will adapt more easily to a serum free medium.
- Always monitor the secretion level of mAb or recombinant protein during the transition from serum to serum-free medium. Some cell lines are adversely affected by serum free medium formulations and may exhibit reduced protein secretion levels.

CDM-HD: CDM-HD Serum Replacement is a chemically defined, protein free serum replacement that permits any basal medium to be used without serum. CDM-HD is designed specifically for the

culture of cells at high density and optimized for use in our hollow fiber bioreactor systems. Secreted products such as monoclonal antibodies and recombinant proteins are free of contaminating proteins from the medium and can be purified using simplified protocols, increasing net yield in many cases. CDM-HD provides lot-to-lot consistency and is an economical replacement for serum. It is available as a dry powder to make up one liter and is used at a concentration of 10%. CDM-HD is animal component free and so contains no agents of unknown origin such as mycoplasma, viruses etc.

Instructions for use: *Reconstitute the contents of one bottle of CDM-HD to make a total volume of 1 liter in distilled water. Sterile filter, do not autoclave. Adjust the pH to 6.8. Add to any basal medium at a concentration of 10%. When using with FiberCell® Systems hollow fiber bioreactors add 10% fetal calf serum to the cell inoculum when loading cells. This permits good attachment of anchorage dependant cells. Switch to CDM-HD after 5 days of culture or when the glucose rate reaches 1 gram per day. No adaptation is required though you may see a reduction in glucose uptake rate for the first day or two of use. CDM-HD is stable for at least 2 months as a concentrate when stored at 4 degrees and away from light.*

Notes:

- *CDM-HD will not require any adaptation but is optimized for cells growing at high density. For best results switch to CDM-HD after cells have reached high density inside the FiberCell® Systems hollow fiber bioreactor, typically after one week of culture or so.*
- *CDM-HD is protein free and does not contain any cell attachment factors. When inoculating cells in medium containing CDM-HD add 5-10% FBS to the cell inoculum only. This will provide the required attachment factors for adherent cells. This is not required for suspension cells though hybridoma cell lines should be treated as adherent cells.*
- *CDM-HD can be used in other culture systems such as spinner culture and roller culture. For these applications add plurionic or some other surfactant.*
- *CDM-HD is protein free. The secreted protein of interest may be the only significant protein present in your cell culture supernatant. You should re-evaluate your purification protocols as entire steps can sometimes be eliminated, increasing yield. Keep in mind that CDM-HD is protein free and contains no ferritin so the free iron levels will be higher than in standard mediums. Pay attention to any chelating agents that may be part of your buffers or purification protocol.*

Flow Rate

- Initial flow rates during the first few days of culture should be set at 18 - 20 After 3 - 4 days or when the glucose rate reaches 150 - 250 mg/day the rate can be increased to 20 - 25.
- At glucose rates > 250 mg/day set the flow rate to 25 or higher.

Inoculation Numbers

How many cells should I inoculate?

- The general rule is to inoculate sufficient cells to cover ~50% of the outer fiber surface area. The number should be based on the average number of cells harvested from a "just confluent" cell culture dish of flask
- Cultures that are "under inoculated" will appear to go into a form of stasis, they do not grow nor do they die. This is a sure sign of an under inoculated culture.
- Some cell types, e.g., lymphocytes and hybridomas, require a critical mass of cells in order to condition the medium. Too few cell inoculated will result in a dead culture.
- The more fastidious cell types will also benefit from the use of conditioned medium in the reservoir bottle. Save the medium from the cell expansion, sterilize by filtration, and supplement the initial medium to a 25 – 50% level.
- Always determine the precise cell number and percent (Trypan Blue exclusion) viability of the cells inoculated. It is difficult to trouble shoot or reproduce a bioreactor culture where the cell inoculum was "a T-225 flask" with unknown viability.
- Cell viability and sterility check.
    - Plate a known number of the cells in a T-25 or 35 mm dish. The next morning perform a visual determination of the fraction of non-attached cells or "floaters". A healthy culture will have a few floaters. However, if >50% of the cells did not attach the bioreactor culture may be compromised.
    - Incubate this dish for 5 - 7 days as a sterility check. Many molds take a week or longer to reach a detectable size.

Can I perform a second inoculation?

- If the culture does not show signs of growth a second inoculation can be performed.
- Increase the inoculum size by 2 - 3 fold to provide a sufficient cell mass to adapt to the bioreactor.
- If the original cell inoculum had poor viability flush the ECS of dead cells and cell debris prior to re-inoculation.

If the culture dies, can I re-use or re-inoculate a cartridge?

- If the culture dies a second inoculation can be performed provided the cartridge is not contaminated.
- Increase the inoculum size by 2 - 3 fold to provide a sufficient cell mass to adapt to the bioreactor if the likely cause was insufficient cell numbers inoculated. Reduced medium volume in the reservoir may also be helpful.
- Always flush the ECS of dead cells and cell debris prior to re-inoculation.

Cartridge Maintenance

What is the correct protocol to maintain the optimum cell number?

- At day 5 - 7 the culture will reach a maxim glucose consumption rate characteristic of that cell line/cartridge/fiber/medium combination.

- The recommended harvest protocol is a single, uni-directional flush of the ECS to remove the ECS medium containing the mAb or recombinant protein. Cells will also be flushed out during harvest.
- Some cell lines will eventually occlude the ECS given sufficient time and minimal ECS flushing.
- Monitor the glucose rate after harvest.
    - If the rate decreases slightly and recovers fully within a day or two the harvest and cell depletion protocol used was correct
    - If the rate decreases substantially, cells must be replaced into the ECS after harvest. This is usually not the case.

How long will my bioreactor cartridge last?

- The all-time record is 560 days for a glioma-based culture.
- Many hybridoma bioreactor cultures will last for several months assuming proper sterile technique and ECS maintenance protocols.
- Tips for long life:

    - Always wipe the Reservoir bottle cap and luer fittings with an ethanol swab during feeding and harvesting.
    - Perform a reverse ultra-filtration harvest (high glucose rate harvest described in the video manual) at least once a week. This helps to keep the pores of the fibers open.
    - Use new luer caps each time you access the ECS via the sideports.
    - Use one medium bottle per culture.
    - Never open a cold medium bottle – the negative pressure inside the bottle may draw in water, condensation and microorganisms.
    - Keep the hood clean and uncluttered
    - Don't rush your bioreactor work!

Can the cartridge be reused?

- The cartridge and/or flow path cannot be autoclaved.
- The cartridge should only be re-used with the same cell line.
- A recommended, but not guaranteed, protocol for reuse is as follows:
    - Fill the ECS with trypsin; incubate 30 min at 37 degrees for 30 min and flush with PBS/pen/strep/fungizone several times.
    - Flush the flowpath with PBS/pen/strep/fungizone.
    - Seal the module in a zip-loc plastic freezer storage bag and store at 4 degrees.
    - Reconstitute the module by replacing the PBS/pen/strep/fungizone in the flowpath and cartridge with complete medium.
    - Perfuse in the incubator at maximum flow rate
    - Change the medium in the ECS and flowpath daily for three day.
    - Inoculate cells as per standard SOP.

Can the cartridge be re-sterilized?

- No. Steam sterilization will damage the cartridge shell and fibers.
- Organic solvents will damage the cartridge shell and fibers.

Trouble Shooting

System Problems

Flowpath won't prime

- Check that the slide clamps on the inlet and outlet are open.
- Check that there are no kinks in the oxygenation loop of tubing.
- Are the Reservoir Bottle inlet tubes submerged in medium?
- Check valve is stuck. Connect a syringe to the flowpath tubing at the cartridge inlet and pull a vacuum.

Pump doesn't work

- Check all connections
- Is the transformer plugged in?
- Check the current in the wall outlet
- Contact FiberCell® Systems

Air bubbles in the flowpath tubing

- Pump the system by hand making sure that the Reservoir Bottle outlet tube is submerged and not pulling in bubbles.
- Purge the system and cartridge by hand pumping.
- Open the Reservoir Cap in the hood to equilibrate the system
- Always use prewarmed medium.

Air bubbles at the cartridge inlet

- Tilt the outlet side of the cartridge up and hand pump the system.
- Always use prewarmed medium.

Air bubbles in the ECS

- This is common early in pre-culture. Continue to pre-culture and check after a few hours.
- In the laminar flow hood flush the bubbles into a syringe with medium and remove.
- Elevate the medium reservoir bottle so that the level of medium is above the level of the ECS in the cartridge.

Cell Death

Cells die after inoculation into the cartridges

- Too short a pre-culture period. Recommended protocol is for 3 medium changes over 3 days.
- Too few cells were inoculated. Check your calculations and the FiberCell® Systems Inoculation Protocols.

- Poor viability of inoculated cells. The viability must be >75%.
- Use conditioned medium at 25 - 50% in the Reservoir Bottle.

Cells die slowly after inoculation

- Lactate levels are allowed to get too high. Change the medium more often.
- ECS is occluded. Flush ECS after harvest to remove excess cells.
- Glutamine has gone bad. Supplement medium with fresh glutamine at 2 mm.

Cells die overnight

- Slide clamp(s) were left in the closed position. Open clamps. Perfuse system for 60 min and perform a lactate determination then and a day later to see if cells are dead.
- Zero glucose consumption means that the cells are dead or in lag phase. If cell viability is below 10% flush ECS and re-inoculate.

Serum

Cells won't adapt to lowered serum

- See FAQ recommendations in the previous section.
- Reduce the rate at which the serum concentration is reduced.
- Some cell lines may not be capable of growth at reduced serum.

Cells won't adapt to serum free medium

- See FAQ recommendations in the previous section.
- Reduce transition to serum free medium rate.
- Some cell lines may not be capable of growth in serum free medium.

Medium Color

Medium is bright orange or colorless

- Lactate concentration is too high. Replace the medium in the Reservoir Bottle.
- Change medium more often

Medium is bright red

- CO_2 level is too low. Check the CO_2 tanks for pressure. Check the lines from the tank to the incubator.
- Check the CO_2 concentration in the incubator with a Fyrite at least once a week.

ECS is yellow but the flowpath medium is red

- Cells, cell debris, cell derived extracellular matrix proteins may have compromised diffusion across the fibers. Flush the cartridge to remove excess cells. Reverse ultra filtration, pulling medium from the flowpath into the ECS, may also help.
- Bacterial or yeast contamination in the ECS. Remove an aliquot and examine microscopically.

Contamination

Cells consume glucose but no lactate is produced

- The system has a bacterial (yellow) or yeast (pink and cloudy) contamination. Check microscopically. If contaminated dispose of immediately. Rinse bottle and cap assembly in a bleach solution. Clean the laminar flow hood thoroughly afterwards. Dispose of all medium used for this bioreactor.
- The cells may be dying. Culture undergoing autolysis may utilize glucose but cannot produce lactate. Check an aliquot of cells from the ECS for viability.

Medium in the reservoir bottle is cloudy and pink

- The system has a bacterial (yellow) or yeast (pink and cloudy) contamination. Check microscopically. If contaminated dispose of immediately. Rinse bottle and cap assembly in a bleach solution. Clean the laminar flow hood thoroughly afterwards. Dispose of all medium used for this bioreactor.

Inlet side of cartridge is occluded with a plug of white material

- Fungal contamination. Mycelia in the perfusing medium have collected at the inlet of the cartridge. If contaminated dispose of immediately. Rinse bottle and cap assembly in a bleach solution. Clean the laminar flow hood thoroughly afterwards. Dispose of all medium used for this bioreactor.

Appendix I – Aseptic Technique

A) Correct sterile technique will ensure a long a productive life for you FiberCell® Systems hollow fiber cell culture cartridge. Shortcuts, suspect medium and careless cell culture techniques will result in a contaminated cartridge that cannot be salvaged.

B) Maintain a clean incubator. Always clean up spilled medium. Disinfect the interior of your incubator on a routine basis with a Rocall/ethanol wash or other appropriate solution.

C) Use clean distilled water with a non-volatile growth inhibitor such as Rocall (benzalkonium chloride) in the humidity pan. A humidified incubator is critical as water vapor can diffuse out of the hollow fiber cartridge oxygenation loop.

D) If supplemented growth medium is used it should be sterility tested before use.

E) The FiberCell® Systems module should be pre-cultured at maximum flow rate for 3-4 days prior to cell inoculation to check for leaks, contamination and to equilibrate the system.

F) Check to insure that all luer fittings are securely tightened.

G) Allow bottles of cell culture medium, serum and supplements to warm to 37°C prior to opening. The reduced pressure inside a cold medium bottle will draw in droplets of liquid and contaminants on opening.

H) Always wipe all luer fitting with an alcohol pad prior to opening. Remember, it is the evaporation of alcohol that sterilizes, not the application so allow time for the alcohol to evaporate.

I) When in doubt, use a new pipette, syringe or bottle of medium. It is less expensive to discard or re-filter a bottle of suspect medium or pipette than to risk a contaminated culture.

J) Always use a large bore needle to draw medium or cells into a syringe. Droplets of medium from the syringe around the luer fittings invites contamination.

K) Use care and common sense.
Hollow fiber cartridges have been continuously maintained for over two years of continuous culture. Following the above guidelines will help insure a long-lived, productive culture.

Appendix II – Suppliers List

Sterile Male/Female Luer Plug	Medex (800) 848-1757 (614) 876-0375 FAX Catalog number #MX-491
Luer Lock Syringes	All major medical distributors 10cc, sterile, individually wrapped Specify syringes with Luer-Lok tips Eg B-D #309604
Lactate Measurement	YSI 2300 STAT Plus Glucose & Lactate Analyzer Yellow Spring Instruments Life Sciences Customer Service P.O. Box 279 Yellow Springs, Ohio 45387 800 659 8895
Glucose Measurement	Accu-chek Advantage Meter (Cat. # 03149137001) Roche Diagnostics Available at most pharmacies and drug stores.
2 and 4 liter medium bottles	United States Plastics Catalog number 72004, 72005 1390 Neubrecht Rd. Lima, Ohio 45801-3196 Phone: 1-800-809-4217 Fax: 1-800-854-5498 www.usplastic.com