

QUICK START GUIDE



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 435-512-8658
Revision 5.2 6/6/2017

TABLE OF CONTENTS

Introduction	2
General Culture Guidelines	3
Reservoir Bottle and Cap Assembly Sterilization	4
Pre-culture	5
Cell Inoculation	9
Daily Maintenance.....	11
Daily maintenance Schedule	15

IMPORTANT! Good Sterile technique is a requirement for working with our system.

This Guide is intended to be an abbreviated instruction manual providing the basics for operating a FiberCell Systems cartridge. Please refer to the FiberCell Video CD Instruction Manual which includes the complete FiberCell Systems User's Manual for more information. If you do not have a copy of the manual, please contact us at (301) 471-1269 or info@FiberCell Systems.com to receive your copy.

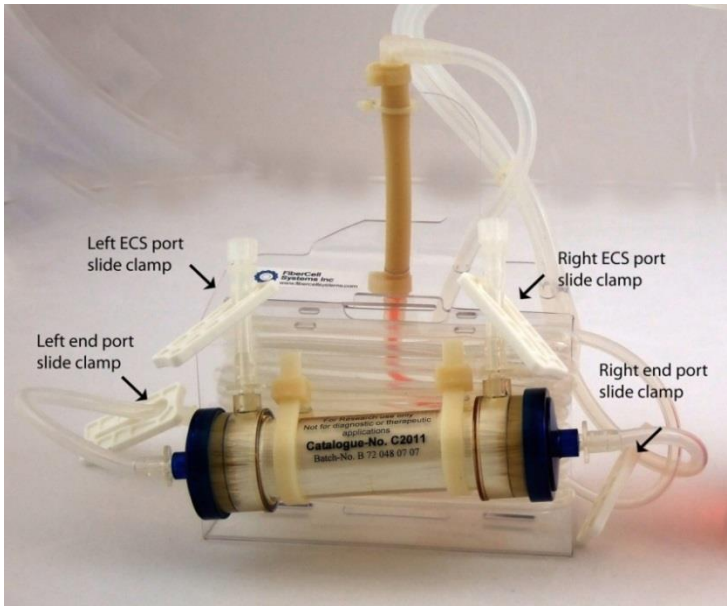


Photo: FiberCell Systems cartridge with ports identified.

INTRODUCTION

Thank you for your purchase of a hollow fiber bioreactor system from FiberCell Systems. A hollow fiber bioreactor cartridge will allow you to culture more cells, produce more protein and antibody at a higher concentration and in a smaller space than is possible with any other culture method. Because the cells are growing at 100X density than other techniques there will be some methods that are counter-intuitive to the ways that you may currently be growing cells.

These products are for laboratory use only. Not for diagnostic or therapeutic use in humans or animals.

FiberCell Systems Technical Support

This Quick Start Guide is to be used in conjunction with the FiberCell Systems Video CD Instruction Manual which provides important visual clues to understanding and operating the system.

Please note that the complete FiberCell Systems User's Manual can be found on the FiberCell Systems Video CD Instruction Manual. Please refer to this manual for detailed instructions on all applications and uses of the FiberCell System.

When in doubt please feel free to contact FiberCell Systems technical support at (301) 471-1269 or 435-512-8658.

GENERAL CULTURE GUIDELINES

Technique

- Correct sterile technique will ensure a long and productive life for your hollow fiber module. Shortcuts, suspect medium, and poor sterile technique may result in contamination.
- Use a needle to draw liquids into syringes. Droplets of medium at the syringe/side port junction invite contamination.
- Disconnect the needle from the syringe after loading and attach syringe directly to the ECS port. This is especially important when working with bacteria.
- Perform all operations in the laminar flow hood. Keep the hood clean. Avoid rapid movements and working directly over the samples.
- Always wear a lab coat and gloves or sterile sleeves. Observe good sterile technique at all times.

Module

After the PBS flush, be sure to pre-culture the module for at least 3 days with two changes of medium prior to cell inoculation.

Cells

- FiberCell Systems Hydrophilic Polysulfone fibers are appropriate for the culture of both adherent and suspension cell lines.
- Cells should be at least 90% viable. Minimize the time between cell harvesting and inoculation into the cartridge.

Media

- Use the same medium used to grow the cells of choice in flask culture. The use of a high glucose (4.5 g/L) medium is strongly recommended therefore the use of low glucose RPMI is to be avoided if possible.
- If serum free medium is desired, perform the adaptation after the cells have reached high density inside the hollow fiber module. Follow the adaptation protocol in the FiberCell Systems User's Manual. For many cell lines including hybridoma, CHO, and recombinant 293 cell lines,

excellent results can be obtained using CDM-HD from FiberCell Systems: no adaptation is required. Please refer to our website or contact FiberCell Systems for more information on CDM-HD. It is much easier to adapt the cells to a serum free medium after the cells have reached a high density inside the cartridge than to do so in a flask or spinner culture.

- Warm media and reagents in a 37°C water bath. Wipe bottles down with alcohol before putting in the laminar flow hood. The reduced pressure inside a cold bottle of medium will draw in air upon opening.
- Pipette, never pour, media and reagents.

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.

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 45mm cap, the tubing is not adjustable.
2. Attach the two pieces of 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 and luers 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.*

NOTE: *There is no directional orientation for the stainless steel tubes. The inlet and outlet tubing may be connected to either luer fitting on the reservoir cap.*



PRE-CULTURE

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

MATERIALS

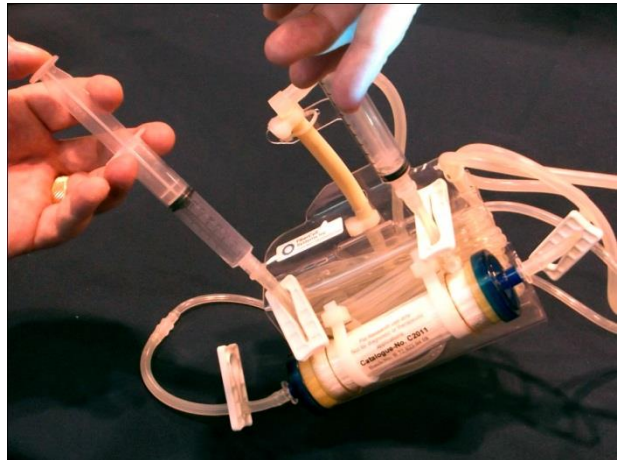
Cells will not be inoculated until several days later. Please have the following materials on hand in the hood prior to starting:

- ✓ Sterile 500 mL bottle of 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 18 gauge needles - for safety blunt end needles are recommended
- ✓ 50 mL conical centrifuge tube filled with 50 mL PBS for filling the ECS
- ✓ 25 mL or 50 mL pipettes
- ✓ If you are using a 500 mL bottle of Gibco PBS you will require the 45 mm reservoir cap

The cartridge should be pre-cultured in the incubator for 24 hours each of 500 mls PBS followed by 24 hours of 125 mL of medium without serum then followed by 125 mL of complete medium for a total of 72 hours

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. Pull 50 mL out of the 500 mL bottle of PBS and place into the 50 mL conical tube, carefully take the reservoir cap out of the autoclave pouch, remove the tin foil on the ends of the tubing and replace PBS bottle cap with the reservoir cap.
2. Attached the tubing from the cartridge to the luer connections on the reservoir cap.
3. Check that the left and right end port slide clamps are in the OPEN position and both left and right ECS ports are closed.
4. 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.
5. 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. Fill a second syringe with 30 mL of PBS, remove needle and connect syringe to the other ECS side port.
4. Open the left and right ECS slide clamps.
5. Inject the PBS into the ECS displacing the air into the other syringe, tilting the right side of the cartridge up. If the ECS is not completely filled with medium, refill syringe and repeat, dislodging all air bubbles.
6. Close the ECS side port clamps, remove the air from the syringes and use the syringes as caps. Remember to use fresh syringes for any subsequent manipulations.
7. Open the left and right end port slide clamps.
8. 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: <http://www.fibercellsystems.com/products/cdm-hd-chemically-defined-high-density-serum-replacement/>

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 side 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. Flow rate should be 20-25.

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

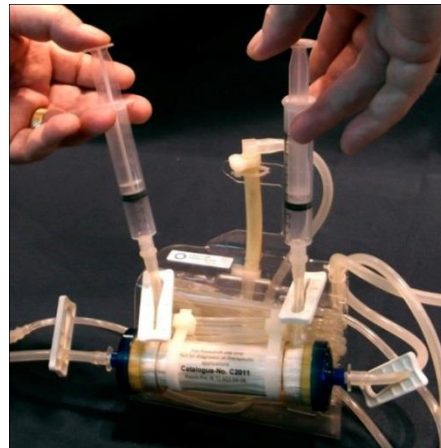
Put on a fresh change of DMEM plus 10% fetal bovine serum, 125 mL. Larger volumes have been used as a convenience to this point however during the initial seeding period it is important that the volume be no more than 125 mL. The volume of media in the reservoir bottle needs to remain proportional to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

You are now ready to inoculate with cells.

CELL INOCULATION

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.
- 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.



Please refer to the FiberCell Systems website, www.fibercellsystems.com for access to training videos.

Procedure for 20 kd MWCO cell inoculation

1. Close the left and right end 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. When using the 5 kd MWCO fiber reduce this volume to 4 mL or less.
5. Attach syringe containing cells to the other ECS port. Open the left and right ECS side port clamps.
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 $\frac{1}{2}$ of the cell suspension to remain in each syringe.
8. Close one of the ECS side port slide clamps. It does not matter which you start with. Open the right end port slide clamp.

9. Crack the reservoir cap by $\frac{1}{2}$ turn. 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.
10. Close the ECS slide clamp and repeat with the opposite syringe, remembering to close ECS ports after expelling cell suspension and opening the left end port slide clamp.
11. Tighten the reservoir cap.
12. Allow the cartridge to sit in the hood for one hour, rotating it 180 degrees after 30 minutes.
13. Leave these syringes on to help guard against contamination.
14. 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. If you do not have a chemical analyzer, we recommend purchasing the Cesco Bioengineering GlucCell, a glucose meter designed for classical media (www.cescobio.com.tw). Be careful not to use a diabetic meter as the chemistry is not compatible with cell culture media.

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 1 L if desired. Once the glucose consumption rate is 1.0 grams per day or higher, harvesting may begin. At this point, CDM-HD may be substituted for serum.

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 the 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 other ECS port. It should be possible to easily get 2 mL through the fiber.
11. Make sure both the ECS side port clamps are closed and end port slide clamps are open.
12. Allow the cartridge to sit in the hood for one hour, rotating it 180 degrees after 30 minutes.
13. Place the cartridge onto the pump and set pump speed to 20-25.

DAILY MAINTENANCE

You may post the Daily Maintenance Schedule (see page 15) in your workspace to keep track of maintenance requirements for your FiberCell Systems.

Harvesting from a hybridoma culture should be performed every other day, for CHO or 293 cell lines producing a recombinant protein, harvesting should be performed every day, if possible. Harvesting from the cartridge is intended to accomplish two things. The first is to harvest the secreted product as concentrated as possible. The second is to control the cell mass -and keep it from getting too high. Removal of dead cells is also important. If there are too many cells in the cartridge then it is possible to exceed the capacity of the system to deliver oxygen. This can drive the cells into anaerobic metabolism. Once the cells are in anaerobic metabolism it can be difficult to get them to recover. The low glucose rate harvest will provide the highest

concentration of product but will not remove many cells. The high glucose rate harvest will remove cells and keep the pores of the fiber open but will dilute the product. The low glucose rate harvest should always be performed first, followed by the high glucose rate harvest if the glucose rate is above 1.5 grams per day, or at least once a week.

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
- ✓ 20 cc 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. Place a new, empty syringe on the right ECS port. 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. This type of harvest is the most concentrated.
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. It is a good idea to pump the media with your fingers to be sure the media is circulating.

High Glucose Rate Harvest

Use this procedure when the glucose rate is 1500 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.

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 for 20kd MWCO High Glucose Rate Harvest

1. Close the left end port slide clamp (right end port remains open).
2. Attach two fresh 20 mL syringes to the ECS side ports.
3. Crack the reservoir bottle cap about 1/4 turn.
4. Open the right ECS port slide clamp.
5. Pull 10 mL gently 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. Making sure both the left and right end port slide clamps are closed, open both ECS port slide clamps.
11. Swish the medium between the two syringes, 2-3 times, gently. The higher the glucose uptake rate, the more swishes you should use.

12. Push all the media into one of the syringes, doesn't matter which one.
13. Close the ECS side 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.

Procedure for 5KD MWCO High Glucose Rate Harvest

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 1 mL of medium into the left syringe.
6. Close the left ECS side port clamp; open the right ECS side port clamp.
7. Pull 1 mL medium into the 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 a 50 mL conical tube and spin down.
13. Re-attach syringes to the ECS side ports. These will serve as end caps.

For a more detailed description of operating your FiberCell Systems Hollow Fiber Bioreactor, please refer to the FiberCell Systems User's Manual, which can be found at our website: www.fibercellsystems.com. For further information please visit our web site at www.fibercellsystems.com or contact FiberCell Systems technical support at (301) 471-1269.

PRE-CULTURE AND DAILY MAINTENANCE SCHEDULE

(Volume in parentheses refer to C2003 and C2018 cartridges)

DAY	PROCEDURE
0	Attach cartridge to 500 ml bottle of PBS, pump manually to fill cartridge, fill ECS with PBS (at this point the cartridge can be left for a week or more before proceeding)
1	Change medium reservoir to 125 mls of basal medium (without serum) and exchange ECS with new medium
2	Change medium reservoir to 125 mls of complete medium and exchange ECS with new medium
3	Change medium reservoir to 125 mls of fresh complete medium. You are now ready to inoculate cells.
4	Inoculate Cells 125 mL media (250 mL media)
5	Check glucose level
6-7	Check glucose, replace medium with 250 mL (500 mL) when 50% of glucose has been consumed
8-9	Check glucose, replace medium with 500 mL (1 L) when 50% of glucose has been consumed
9-10	Check glucose, replace medium with 1,000 mL (2 L) when 50% of glucose has been consumed
10-12+	Check glucose, replace medium when 50% of glucose has been consumed. Harvest Antibody from ECS every other day. Harvest proteins every day.

