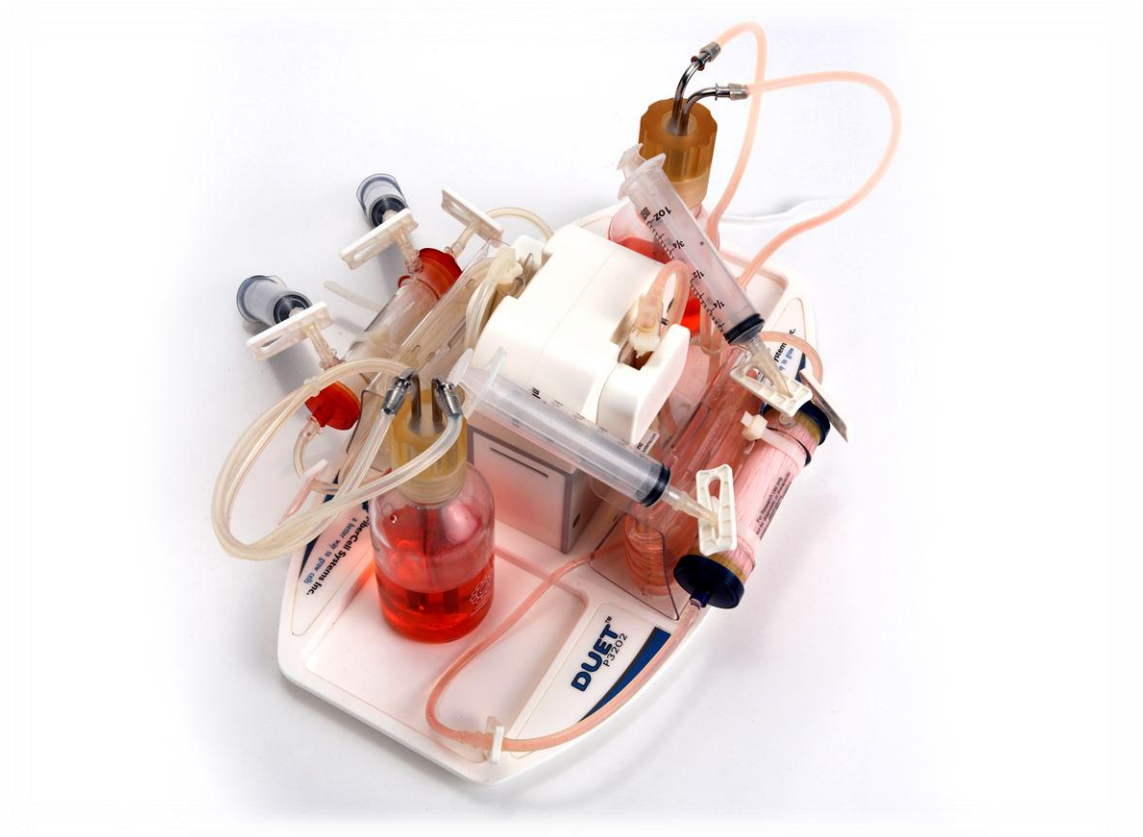

Quick Start Guide

FiberCell Systems Inc.
a better way to grow cells

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Table of Contents:

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@fibercellsystems.com to receive your copy.

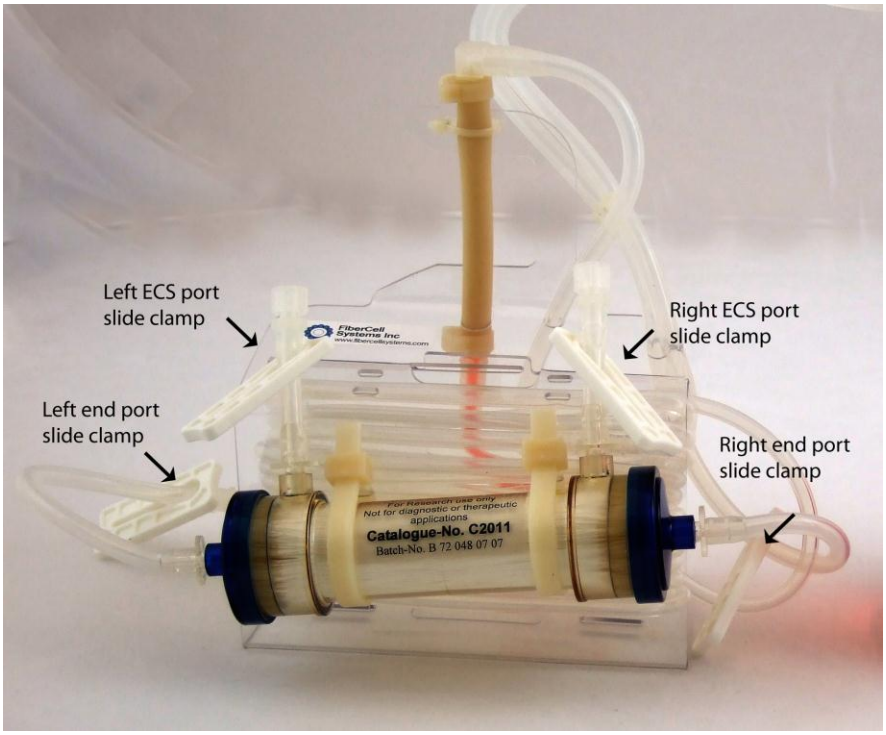


Photo: FiberCell® Systems cartridge with ports identified.

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.

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 bacterial organisms.
- 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^{0}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 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: 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 PBS
- ✓ FiberCell® Systems culture module
- ✓ FiberCell® Systems reservoir cap, autoclaved, with tubing attached
- ✓ 20cc 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 50mL pipettes
- ✓ Sterile 250 mL plastic Nalgene bottle (38 mm cap) or sterile 250 mL glass bottle with black phenolic cap (33mm)
- ✓ If you are using a 500mL bottle of Gibco PBS you will require the 45mm 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/products_cdmhd.htm

First Media Change

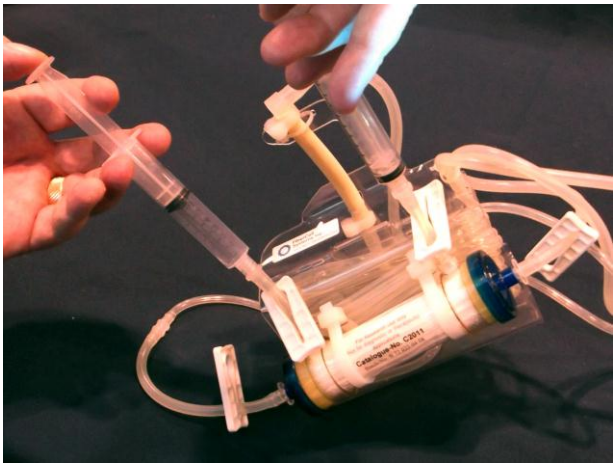
Procedure

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 125mL. The volume of media in the reservoir bottle needs to remain proportional to the number of cells in the cartridge.

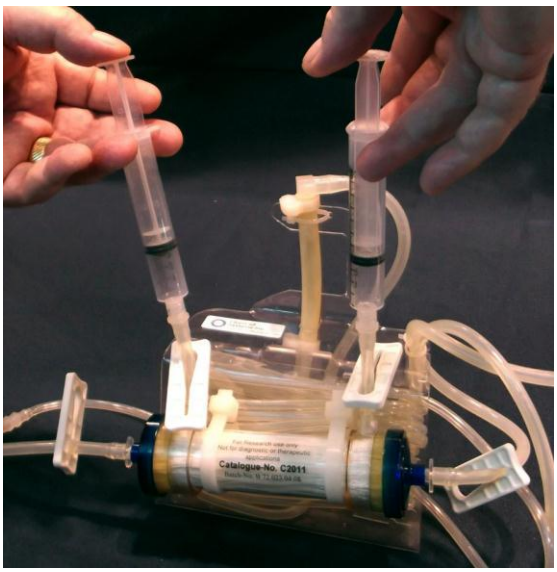
You are now ready to inoculate with cells.

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 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. When using the 5kd MWCO fiber reduce this volume to 4 mL or less.

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 Accucheck Cat#03149137001, or equivalent chemistry.

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

Daily Maintenance

You may post the Daily Maintenance Schedule (see page 12) in your workspace to keep track of maintenance requirements for your FiberCell® System.

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.

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® 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. 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.
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.

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.

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.

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a better way to grow cells

Daily Maintenance Schedule

(Volume in parentheses refer to C2003 and C2018 cartridges)

Day	**Procedure**
0	Inoculate Cells 125 mL media *(*250 mL media*)*
1	Check glucose level
2-3	Check glucose, replace medium with *250 mL (500* mL*)* when 50% of glucose has been consumed
4-5	Check glucose, replace medium with *500 mL (1 liter)* when 50% of glucose has been consumed
5-7	Check glucose, replace medium with 1*,000 mL (2 liters)* when 50% of glucose has been consumed
8-10+	Check glucose, replace medium when 50% of glucose has been consumed. Harvest Antibody from ECS every other day. Harvest proteins every day.