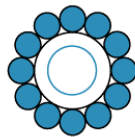


# Quick Start Guide

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**FiberCell Systems Inc.**  
a better way to grow cells

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**IMPORTANT! Good Sterilization 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](mailto:info@fibercellsystems.com) to receive your copy.

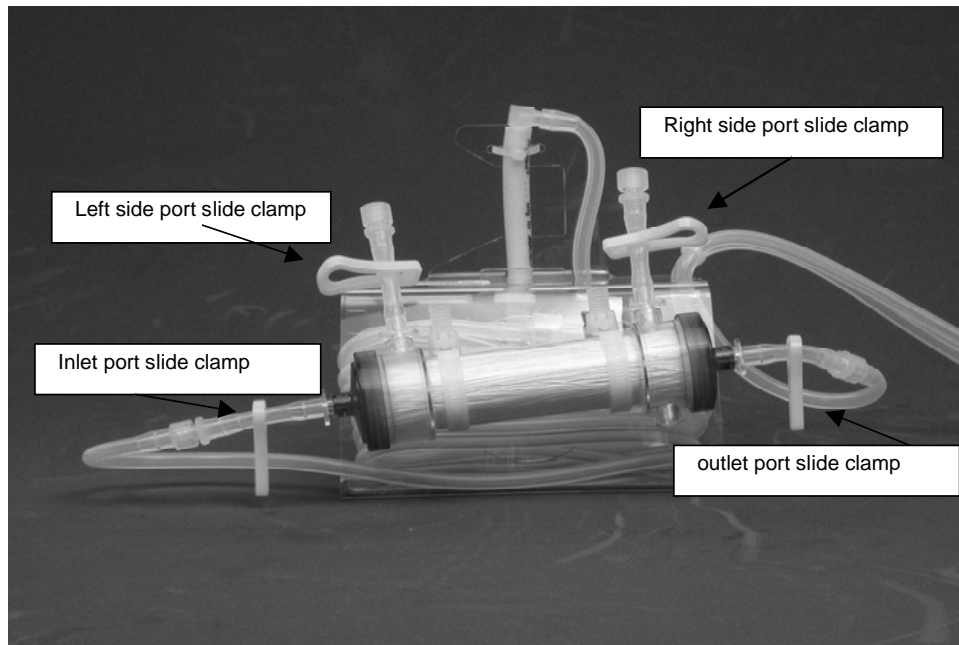


Photo: FiberCell Systems cartridge with ports identified.

## 1) Introduction and General Guidelines

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
- Perform all operations in the laminar flow hood. Keep the hood clean. Avoid rapid movements and working directly over the samples. If necessary, open the hood front to be sure to allow time for the air inside the hood to completely exchange.

### Module

- After the PBS flush, be sure to pre-culture the module for at least 3 days with three 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 grams/liter) 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 hybridoma, CHO, and recombinant 293 cell lines, excellent results can be obtained using CDM-HD from FiberCell Systems. 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.
- Always allow bottles of medium and other additives to warm to room temperature before opening. The reduced pressure inside a cold bottle of medium will draw in air and liquid upon opening.

## 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) (60mls for larger cartridges)
- Alcohol pads
- Spray bottle containing 70% ethanol
- Large bore needles
- 50ml conical centrifuge tubes
- 25ml or 50ml pipettes
- Sterile 250 ml plastic Nalgene bottle (38mm cap) or sterile 250 ml glass bottle with black phenolic cap.

## 2) Reservoir Bottle and Cap Assembly Sterilization

- Attach the reservoir cap tubing supplied with each cartridge to the fittings on the reservoir cap.
- Cover the inlet and outlet luer fittings with autoclave paper or aluminum foil and secure with autoclave tape.
- Place the reservoir cap assembly into an autoclave bag.
- Autoclave the reservoir cap at 120 – 130 °C for 45- 60 min.
- 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:

- Take the reservoir cap out of the autoclave bag and place into a sterile bottle.
- Remove the FiberCell Systems module (with flow path) from the sterile package. All of the open ends are sealed with luer caps.
- 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.*

## 3) Pre-culture

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

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

- (1) remove the wetting agent from the fibers,
- (2) equilibrate the system with growth medium and serum proteins,
- (3) verify that the system is leak free.
- (4) provide a sterility check.

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

### Prime and fill the cartridge with PBS

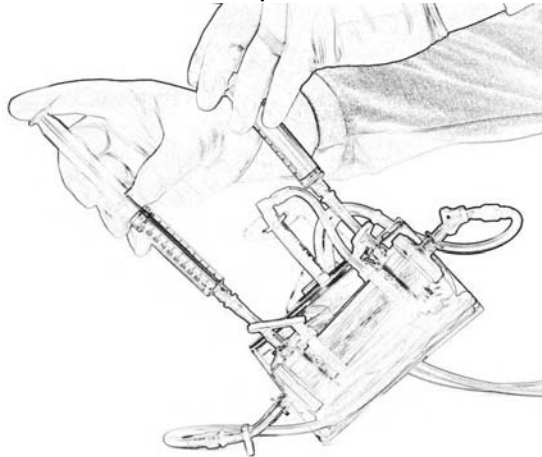
- Check that the inlet and outlet slide clamps are in the OPEN position.
- Using a pipette transfer 200 mls of PBS into the reservoir bottle. Alternatively you can simply attach the reservoir cap directly to a 500 ml bottle of sterile PBS.
- Perfuse medium through the flowpath circuit by pumping the compression tubing with your fingers until the circuit is filled and no bubbles come from the stainless steel tubing inside the bottle.
- 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.

### Fill the ECS with PBS

- Close the inlet and outlet slide clamps on the cartridge to isolate the bioreactor from the flowpath.
- Open the sideport slide clamps.
- Attach a sterile syringe (20– 60 ml depending on the bioreactor size) to one sideport.
- Add ~50 ml of PBS to a 50 ml conical centrifuge tube.
- Fill a second syringe with PBS using a large gauge needle and connect it to the other sideport.
- 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.
- Close the sideport clamps, remove and air from the syringes and use them as caps. Remember to use fresh syringes for any subsequent manipulations.
- Remember to use the alcohol swabs to clean up any medium on the caps or

bioreactor. Whenever the side ports are open be sure to have the slide clamps closed, this will prevent any excess medium from collecting on the fittings.

- Open the inlet and outlet slide clamps.



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.

Place the cartridge onto the Duet and run PBS through the system at a moderate flow rate for a minimum of 24 hours.

After this 24 hour-flush, there will be three 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, a fresh change of medium for cell inoculation. Serum free media works very well with the FiberCell Systems hollow fiber bioreactor. Please contact FiberCell Systems for instructions on adaptation to serum free media inside the cartridge.

## First Media Change

- This first liquid change will be using classical media/serum free media. Screw on a 500mL bottle of media, filled to only 125mL.
- Change the medium in the ECS by filling a 20ml syringe with the new medium and attaching it to the left side port. Place an empty syringe onto the left side port.
- Tilt the cartridge up on the right side and exchange the medium by slowly pushing the new medium in from the bottom and floating the old medium out the right side port by putting on a 20mL media-filled syringe on the left upper luer connection, and pulling the PBS out using an empty syringe attached to the right upper port.
- Remove the PBS from the right syringe and reattach it to the side port.

- Let the media circulate for a minimum of 24 hours, placing the system back in the incubator. **Remember to open the left end port slide clamp!**

## Second Media Change

- If using a basal medium, change out the DMEM for DMEM plus 10% fetal bovine serum. If using serum free medium just make the change using the serum free media, using above protocol Allow this to circulate for 24 hours.

## Final Liquid Change

- Put on a fresh change of DMEM plus 10% fetal bovine serum, or serum free media, 125mL

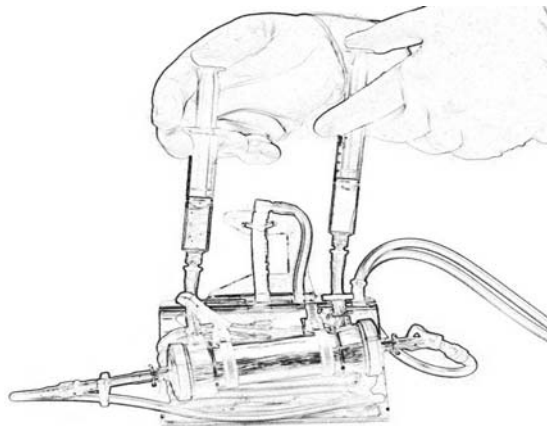
You are now ready to inoculate with cells.

## 4) 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.*

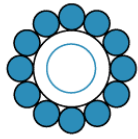


- Close the inlet and outlet ports of the bioreactor.
- Remove one sideport luer cap, remembering to spray with alcohol and wipe, and attach a disposable syringe. If you have been using syringes as caps, use a fresh syringe.
- Fill a second syringe with 20-30mL of cell culture suspension. When using the 5kd MWCO fiber reduce this volume to 10 mls or less.
- Remove medium transport needle and attach syringe to the other sideport luer fitting.
- Displace the preculture medium into the other syringe with the cell suspension. Push gently to avoid creating bubbles or foam.
- 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.
- Crack the reservoir cap by ½ turn. Close one side 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.
- Close the slide clamp and repeat with the opposite syringe.
- Tighten the reservoir cap.
- Allow the cartridge to sit in the hood for one hour, rotating it 180 degrees after 30 minutes.
- Leave these syringes on to help guard against contamination.
- Place the cartridge onto the pump and begin flow.

Change to a 250mL bottle of fresh serum free or serum free media when the glucose has been depleted by half. This will generally be when the glucose level has reached 2 grams per liter. You can purchase an inexpensive glucometer (the kind that diabetics use) at most any drug store.

## I) Daily Maintenance

You may post the following Daily Maintenance Schedule in your workspace to keep track of maintenance requirements for your FiberCell System.



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a better way to grow cells

## Daily Maintenance Schedule

(volume in parentheses refer to C2003 and C2018 cartridges)

<u>Day</u>	<u>Procedure</u>
0	Inoculate Cells 125mls media (250mls media)
1	Check glucose level
2-3	Check glucose, replace medium with 250mls (500mls) when 50% of glucose has been consumed
4-5	Check glucose, replace medium with 500mls (1 liter) when 50% of glucose has been consumed
5-7	Check glucose, replace medium with 1,000mls (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.

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.fibercellsystems.com](http://www.fibercellsystems.com).

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

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