

# Quick Start Guide

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## TABLE OF CONTENTS

Introduction.....	2
General Culture Guidelines .....	3
Reservoir Bottle And Cap Assembly Sterilization.....	4
Pre-Culture .....	5
Cell Inoculation .....	8
Daily Maintenance.....	10
Pre-Culture And Daily Maintenance Schedule .....	13
Glucose Monitoring .....	14
Measurement Of Glucose And The Glucose Uptake Rate .....	14
Starting A Productive Culture In A Fibercell Systems Bioreactor .....	16

**IMPORTANT! Good Sterile technique is a Requirement for working with our system. If you need assistance, we recommend reading; “Helpful Hints for Better Aseptic Technique” ([click here to download the pdf](#)).**

This Guide is intended to be an abbreviated instruction manual providing the basics for operating a FiberCell Systems cartridge. Please refer to the FiberCell Systems Video USB Manual which includes the complete FiberCell Systems User’s Manual for more information. If you do not have a copy of the manual, please call us at 301-471-1269 or 435-512-8658, or email us at [info@fibercellsystems.com](mailto:info@fibercellsystems.com) to receive your copy.

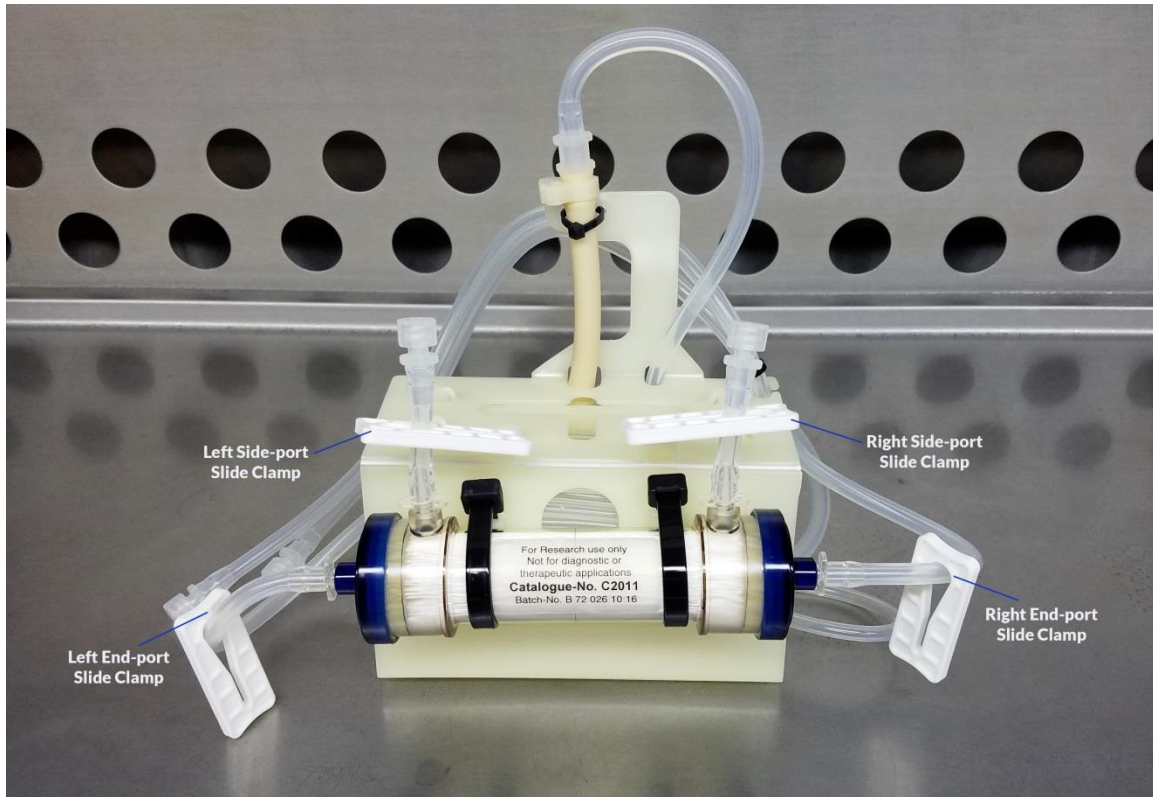


Photo: FiberCell Systems cartridge with clamps identified.

## 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 and produce more protein and antibodies at a higher concentration in a smaller space than possible with any other cell culture method. Because the cells are growing at 100 X greater density than other techniques, there will be some methods described here that are counter-intuitive to the ways you may be currently growing cells.

This Quick Start Guide is to be used in conjunction with the FiberCell Systems Video USB Instruction Manual which provides important visual clues to understanding and operating the system. They can be found here: <https://www.youtube.com/user/fibercellsystems>

## 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.
- 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.
- Replace syringes with fresh ones whenever the cartridge has come out of the laminar flow hood.

### Module

After the PBS flush, be sure to pre-culture the module for two changes of medium, each change a minimum of 24 hours 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

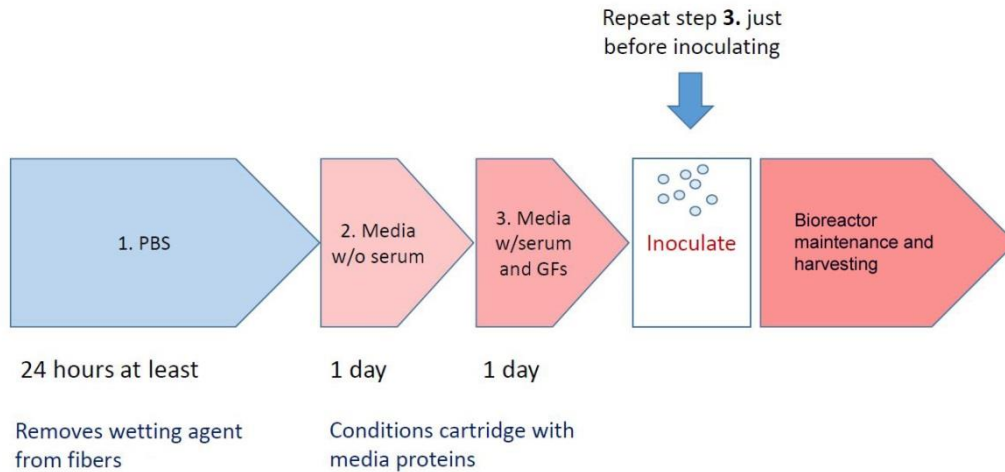
Each cartridge comes with two short pieces of tubing in the outer bag. Being careful not to tear the inner bag, remove the two pieces of tubing. These will be connected to the cap before autoclaving.

1. Hold the reservoir cap up to a sterile 250 mL Nalgene bottle. The stainless steel tubes should reach within a 1/2 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 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.

## Quick Start Outline



## 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
- ✓ 60cc and 20 cc sterile syringes (luer-lock)
- ✓ Alcohol pads
- ✓ Spray bottle containing 70% ethanol
- ✓ Large 14-18 gauge needles - for safety blunt end needles are recommended
- ✓ 50 mL sterile conical centrifuge tube filled with 50 mL PBS for filling the ECS
- ✓ 25 mL or 50 mL pipettes
- ✓ If you are using Gibco media you will require the 45 mm reservoir cap

The cartridge should be pre-cultured in the incubator for 24 hours each of 500 mL 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. Attach 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 to the right ECS side port.
3. Fill a second syringe with 20 mL of PBS, remove needle and connect syringe to the left 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 any air from the syringes and use the syringes as caps. Use fresh syringes for 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 setting 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:

- Use the alcohol swabs to clean up any medium on the luer fittings or cartridge.

- Always have either a syringe or cap on the ECS side ports, or the slide clamps closed to prevent 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 of basal medium without serum or growth factors (or serum free medium) and then a second change of 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 125 mL 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 setting should be 20-25 on the controller.



## 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. Exchange the medium in the ECS as well.

## 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.

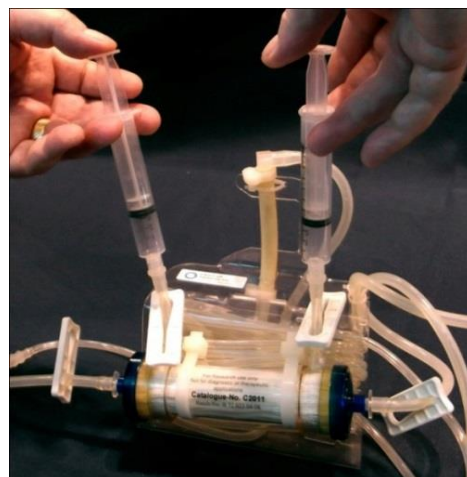
**You are now ready to inoculate with cells.**

## 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 here:

- 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](http://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.
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  $\frac{1}{4}$  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. Flow setting.

Please refer to page 14, "Measurement of Glucose and the Glucose Uptake Rate" for information on glucose monitoring.

## Procedure for 5 kD MWCO Cell Inoculation

1. Close end port slide clamps.
2. Attach a fresh 20 mL syringe to the left ECS side port. Attach an empty 20 mL syringe to right ECS side port with plunger pulled back so it is full of air.
3. Open ECS side port slide clamps.
4. Tilt cartridge and withdraw medium from ECS into the 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. Expel remaining air from right ECS port syringe and re-attach.
6. Resuspend cells to be inoculated in the volume removed plus 2 mL (12-14 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. Crack open reservoir cap  $\frac{1}{4}$  turn. 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. Gentle, steady pressure.
11. Make sure both the ECS side port clamps are closed and end port slide clamps are open. Tighten the reservoir cap.
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 13) 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, 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)
- ✓ 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 in pre-culture section). 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 which indicates that 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)
- ✓ Alcohol pads
- ✓ Spray bottle containing 70% ethanol or isopropyl alcohol

## Procedure for 20 kD 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. Tighten the reservoir cap.

### **Procedure for 5 kD 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 (right), the other empty with the plunger all the way down (left).
3. Open the left ECS side port slide clamp.
4. Open the reservoir bottle cap  $\frac{1}{4}$  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 right 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 of 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. Tighten the reservoir cap.
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](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.

## 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 mL of basal medium (without serum) and exchange ECS with new medium
2	Change medium reservoir to 125 mL of complete medium and exchange ECS with new medium
3	Change medium reservoir to 125 mL 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 recombinant proteins every day.

## GLUCOSE MONITORING

### Measurement of Glucose and the Glucose Uptake Rate

Measurement of glucose and calculating the glucose uptake rate is critically important in the maintenance of a hollow fiber bioreactor. It is difficult to visualize the cells, though small samples can be pulled out and placed into a flask for visualization and as a check for proliferative capability. However, when taking a sample, the non-viable cells will be preferentially collected as they are not adherent to the fiber. This sample will not reflect the overall status of the cartridge. Measurement of the glucose can tell us two things:

1. **The total amount of glucose in the medium** tells us when it is time to change the medium. We want to maintain culture conditions in a homeostatic manner, which means changing the medium before the pH changes, and the color of the medium visibly changes. The medium should be changed when the measured glucose is 50% or less of the original concentration. For high glucose DMEM, which has a starting glucose of 4.5 grams per liter, you will want to change the medium when it is 2.25 grams per liter or less. This guideline applies to most media. If you are not sure of the starting glucose check with the manufacturer of the medium.
2. **The glucose uptake rate**, i.e. the amount of glucose consumed by the cartridge in 24 hours indicates indirectly how many cells are in the cartridge, and how healthy they are. If the rate has plateaued then the cells are in a steady state, if the rate is increasing then the cells are proliferating. If the glucose uptake rate drops then cell number is reduced, cells are dying or something else is going on inside the bioreactor. We know empirically that a glucose uptake rate of 1 gram per day corresponds to approximately  $1 \times 10^9$  cells. The ideal glucose uptake rate for the C2011 and C2008 cartridges is between 1-1.5 grams per day. For the C2018 and C2003 cartridges is it between 2-6 grams per day. It is important to keep the glucose uptake rate below 2 grams per day in the C2011 and C2008 cartridges. In some cases, this indicates that there are too many cells in the cartridge, and they may go into anaerobic metabolism. The glucose uptake rate indicates which type of harvest should be performed, low glucose rate or high glucose rate, and the approximate number of cells you will want to remove.

Glucose uptake rate is calculated by taking the starting glucose measurement and subtracting from it the glucose measurement 24 hours later. Since the rate is dependent upon the size of the reservoir bottle you multiply the drop in glucose in grams by the volume of media in the reservoir

bottle. For example, if there are 250 mL in the reservoir bottle multiply by .25 or 25%.

(Glucose1-Glucose2) X reservoir bottle mL.

If more than one day has elapsed, divide this by the number of days.

For example:

- Starting glucose 4.5 grams per liter
- Measure glucose 24 hours later 2.7 grams per liter
- Reservoir bottle 200 mL

$$4.5 - 2.7 = 1.8$$

$$1.8 \times .2 = .36$$

Glucose uptake rate is 360 mg per day.

Media changes should be performed when the glucose has been depleted by half. This will be when the glucose level has reached 2 grams per liter or less with a medium such as DMEM. If you do not have a chemical analyzer, we recommend purchasing the GlucCell, a glucose meter designed specifically for cell culture media. <https://chemglass.com/glucell-glucose-monitoring-system>. Newer diabetic glucose meters are not compatible with cell culture media.

When the glucose consumption rate is 1.0 grams per day or higher, harvesting may begin. At this glucose rate the cells are at high density and CDM-HD may be substituted for serum. At a glucose rate of less than 1 gram per day only a low glucose rate harvest should be performed. Between 1-2 grams per day a low glucose rate harvest should be performed, followed by a high glucose rate harvest. If you have any questions please feel free to contact FiberCell Systems at [info@fibercellsystems.com](mailto:info@fibercellsystems.com).



*GlucCell classical media glucose monitor or chemical analyzer for measuring glucose. Diabetic test strips will not work with classical media.*



## STARTING A PRODUCTIVE CULTURE IN A FIBERCELL SYSTEMS BIOREACTOR

TO DOWNLOAD A BLANK COPY OF THIS CHART FOR USE, VISIT:

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

Cartridge            C2011  
Media                DMEM/High with 10% FBS  
Glucose             4.5 g/L (450 mg/dL) (5.2 g/L with 10% CDM-HD)

Day	Time	Glucose (mg/dL)	Reservoir Volume (mL)	Glucose Uptake Rate (GUR) (mg/24hr)	Media Change (if any)	Harvest (Y/N)	Harvest Type (High=H) (Low=L)	Media Type
0	2:30PM	450	125					
1	2:30PM	276	125	217	fresh media 250 mL	no		DMEM/ High-7.5% FBS
2	2:30PM	334	250	290	no			
3	2:30PM	190	250	360	fresh media 500 mL	no		DMEM/ High-5% FBS
4	2:30PM	310	500	700		no		
5	2:30PM	175	500	675	fresh media 1L			
6	2:30PM	330	1000	1200		no		
7	2:30PM	205	1000	1250	Fresh 1 liter 10% CDM-HD	yes	low and high	Note, starting glucose is now
8	2:30AM	390	1000	1400		no		5.2 grams per liter.
9	2:30AM	220	1000	1700	Fresh 1 liter 10% CDM-HD	yes	low and high	