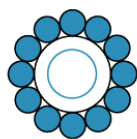
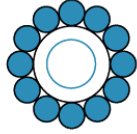


Quick Start Guide



FiberCell Systems Inc.
a better way to grow cells

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

1) 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 (approximately 100x greater than other techniques); produce more protein and antibodies at a higher concentration and in a smaller space than is possible with any other culture method.

Fundamental concepts used for other methods of cell culture are not necessarily appropriate when using our system so it is important that you familiarize yourself with our process. Please be sure to watch the instructional videos and read the FiberCell Systems User's Manual that are included in the FiberCell Systems Video & Reference Materials CD prior to use. When in doubt please feel free to contact FiberCell Systems technical support at (301) 471-1269. You may also visit our website at www.fibercellsystems.com for more information.

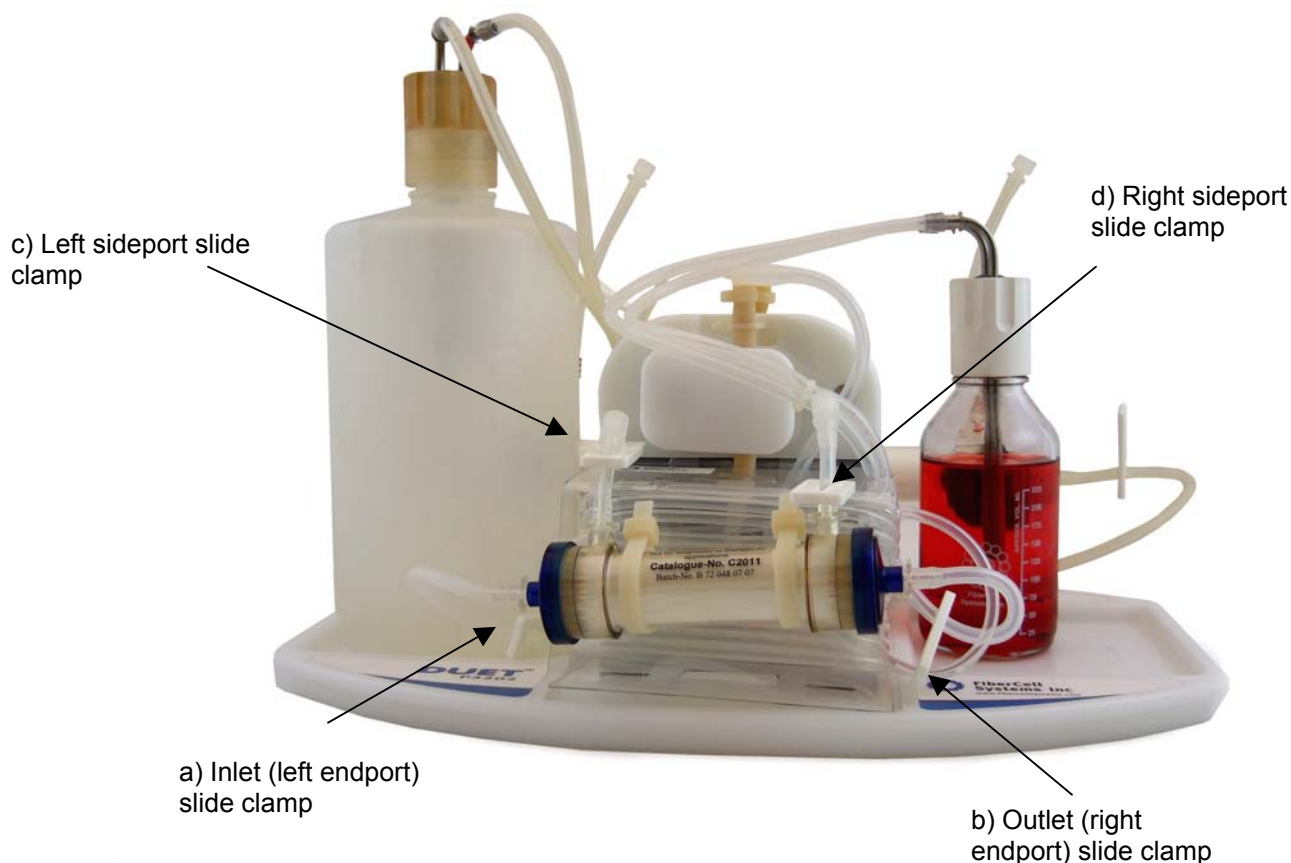


Photo: FiberCell Systems cartridge with ports identified.

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

Culture Guidelines

IMPORTANT: THERE IS ALMOST NO MISTAKE THAT CANNOT BE FIXED EXCEPT FOR CONTAMINATION! GOOD STERILE TECHNIQUE IS A MUST!

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. Work deep inside the hood. Always wear a lab coat and follow all guidelines for good sterile technique when working in a hood. 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. Minimum times are suggested. Longer times can be used except for the last step in which serum is introduced. Because of the nature of serum that step should last no more than 48 hours

Cells:

- FiberCell Hydrophilic Polysulfone fibers are appropriate for the culture of both adherent and suspension cell lines. It is the only hollow fiber system optimized for suspension cells
- 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. Serum free mediums and CDM HD generally do not contain attachment factors so the use of serum in at least the cell inoculum is recommended. Follow the adaptation protocol in the FiberCell Manual. For hybridoma, CHO and recombinant 293 cell lines excellent results can be obtained using CDM HD from FiberCell systems. No adaptation to CDM HD is required though there may be a short lag phase. 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. This is defined as a consumption of glucose of 1 gram per day or more.
- 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.
 - FiberCell Systems always recommends the use of antibiotics such as penn/strep at standard concentrations unless there is a compelling reason to not use an antibiotic. This helps to protect the cartridge from contamination.

2) Pre-Culture Protocol

(Please view the “Setting up a Cartridge” video on the video CD.)

Purpose

The purposes of pre-culturing the cartridges are the following:

- Remove the wetting agent from the fibers
- Equilibrate the system with growth medium and serum proteins
- Verify that the system is leak free before cells are introduced
- Sterility check

Equipment Preparation

RESERVOIR BOTTLE AND CAP ASSEMBLY STERILIZATION

*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.



- Step 1 Attach the reservoir cap tubing supplied with each cartridge to the fittings on the reservoir cap.
- Step 2 Cover the inlet and outlet luer fittings with autoclave paper or aluminum foil and secure with autoclave tape.
- Step 3 Place the reservoir cap assembly into an autoclave bag.
- Step 4 Autoclave the reservoir cap at 120 –130 °C for 45- 60 min. dry cycle.
*Note: 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.*

- Step 5 Remove cap assembly from autoclave and place in laminar flow hood.
- Step 6 Using aseptic technique (please see video) remove module (with flow path) from the sterile package. (All open ends are sealed with luer caps).
- Step 7 Connect the module inlet and outlet tubing to the two luer fittings on the reservoir bottle cap.

Pre-Culturing the Module

The Extra-capillary space (ECS) is defined as the area that is outside the fibers and inside the cartridge. The ECS is accessed through the side port, ports C and D. The space inside the fibers and contiguous with the reservoir bottle is defined as the intra-capillary space or ICS. Please view the “Priming and filling ECS” video on the video CD.

The system will be pre-cultured in the incubator for 72 hours with three liquid changes.

Prime and Fill Cartridge with PBS

EQUIPMENT AND MATERIALS

- FiberCell Module
- Water bath
- FiberCell reservoir cap, autoclaved, with tubing attached.
- 20cc sterile syringes (luer-lock) (60cc for larger cartridges)
- Alcohol pads
- Spray bottle containing 70% ethanol
- Large bore needles (16 or 18 gauge) or cannula
- 50 mL conical centrifuge tubes
- 25 mL or 50 mL pipettes
- Sterile 250 mL plastic Nalgene bottle (38mm cap) or sterile 250mL glass bottle with black phenolic cap.
- Sterile PBS

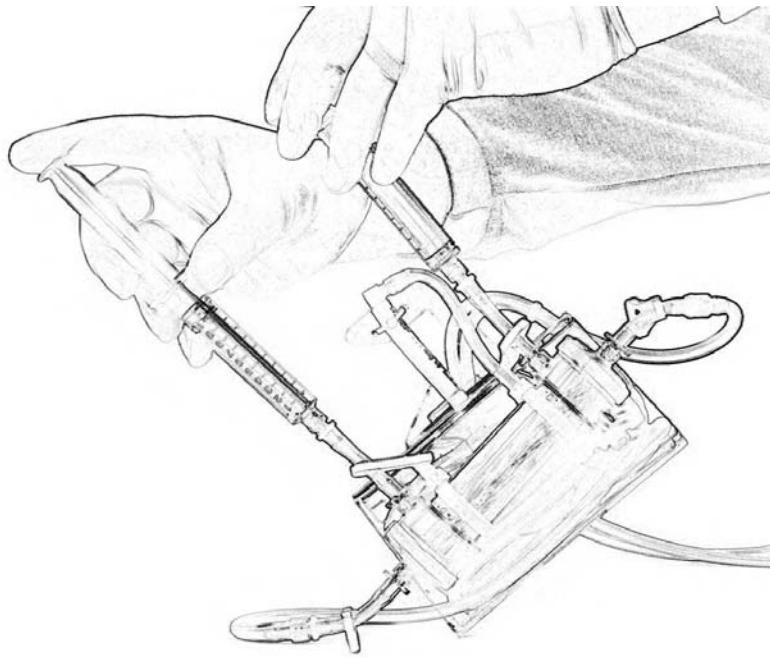
PROCEDURE

- Step 1 Warm all liquids in 37° water bath before use. Be sure to spray bottles with 70% ethanol before setting in clean laminar flow hood.
- Step 2 Check that the (A) inlet and (B) outlet slide clamps are in the OPEN position.
- Step 3 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.

- Step 4 Perfuse medium through the flow path 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.
- Step 5 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

- Step 6 Close the (A) inlet and (B) outlet slide clamps on the cartridge to isolate the bioreactor from the flow path.
- Step 7 Open the (C and D) side port slide clamps. Always spray with alcohol or ethanol and then wipe the side ports with an alcohol swab and allow to dry each time before opening (see video).
- Step 8 Remove cap and attach a sterile syringe (20– 60 ml depending on the bioreactor size) to one side port.
- Step 9 Add ~50 ml of PBS to a 50 ml conical centrifuge tube.
- Step 10 Fill a second syringe with PBS (20 ml for medium sized cartridges, 60 ml for large sized cartridges) using a large gauge needle and connect it to the other side port.
- Step 11 Inject the PBS into the ECS displacing the air into the other syringe.
- Step 12 If the ECS is not completely filled with medium, repeat, dislodging all air bubbles.
- Step 13 Close the (C and D) side port clamps, remove and air from the syringes and replace them on the side ports and use them as caps. Remember to use fresh syringes for any subsequent manipulations and 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.
- Step 14 Open the (A) inlet and (B) outlet slide clamps



Note * 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.

- Step 15 Place the cartridge onto the Duet and run PBS through the system at a moderate flow rate (10-15) for a minimum of 24 hours. This completes the first introduction of sterile liquid into the cartridge.

First Media Change

EQUIPMENT AND MATERIALS

- FiberCell module.
- Waterbath
- 500mL bottle filled with 125mL classical media.
- 20mL syringes.
- 50mL sterile conical tube filled with classical media

PROCEDURE

- Step 1 Place media, syringes, and hollow fiber module in clean laminar flow hood.
- Step 2 Attach the 125mL media to the reservoir bottle.

- Step 3 Change the medium in the ECS by filling a 20mL syringe (60 ml for large cartridge) with the new medium from the 50mL conical tube, and attaching it to the (C) left side port. Place an empty syringe onto the (D) right side port.
- Step 4 Tilt the cartridge up on the right side and exchange the medium by slowly pushing the new medium in from (C) left side port and pulling the PBS out through the empty syringe on the (D) right side port.
- Step 5 Remove the PBS from the right syringe (D) and reattach it to the side port.
- Step 6 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

Follow directions for first media change but change the DMEM for DMEM containing 10% FBS. Exchange the PBS in the ECS with medium by following the same protocol that was used to fill it. Refer to the video. As a general rule you always want the same composition medium in the reservoir bottle as in the ECS. Allow to circulate for 24 hours.

Final Media Change

Put on a reservoir bottle containing a fresh change of DMEM plus 10% FBS, 125mL and exchange the medium in the ECS with the same fresh medium as well. At this time you should also add any other additives that you might wish to use such as cytokines, growth factors, selection agents. Etc.

You are now ready to inoculate with cells.

3) Cell Inoculation

(Please view the “Loading Cells” video on the video CD.)

EQUIPMENT AND MATERIALS

- FiberCell module, pre-cultured
- Waterbath
- Cells
- 20cc sterile syringes (luer-lock) (60cc for larger cartridges)
- Alcohol pads
- Spray bottle containing 70% ethanol or isopropyl alcohol
- Large bore needles
- 50mL conical centrifuge tubes
- 25mL or 50 mL pipettes
- Cell culture media of choice
- Roche Accucheck glucometer or equivalent and test strips

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;

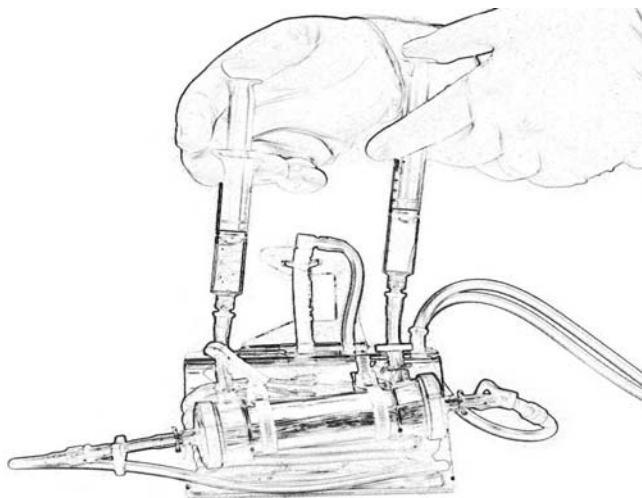
Hybridomas and suspension cells: a minimum of 10^8 total viable cells.

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 for the medium sized cartridges and 20 or more for the large sized cartridges. Fewer cells can be inoculated if the starting volume in the reservoir bottle is reduced and it is understood that it may take a few extra days for the culture to reach high cell density.

The gross filtration rate of the 20kd MWCO fibers (C2011 and C2018) is much higher than that of the 5kd MWCO fibers (C2008 and C2003). It is difficult to ultra-filter larger volumes of medium containing cells through the 5kd fibers. For this reason the volume of the cell inoculum for the 5kd fibers should be much smaller, 5 mls or less for the C2008 and 15 mls or less for the C2003. Some resistance is to be expected. Slow steady pressure will ensure smooth ultra filtration of the cell inoculum in these cartridges.

Serum free mediums and CDM HD generally do not contain attachment factors. Although it is recommended to adapt the cells to a serum free medium inside the cartridge once they have reached high density (defined as 1 gram per day of glucose consumed, 2-4 grams per day for the larger cartridges) cells can be inoculated directly in serum free medium. For most consistent results it is recommended that 5-10% FBS be added to the cell inoculum to facilitate attachment of the cells.

Please refer to the FiberCell Video & Reference Materials CD for visual instructions



PROCEDURE

- Step 1 Close the inlet (A) and outlet (B) ports of the bioreactor.
- Step 2 Remove one side port luer cap (C or D), remembering to spray with alcohol and wipe, and attach a disposable syringe. If you have been using syringes as caps, use a fresh syringe.
- Step 3 Fill a second syringe with 20-30mL of cell culture suspension using a needle or cannula attached to syringe to draw up the liquid. When using the 5kd MWCO fiber reduce this volume to 10 mL or less.
- Step 4 Remove medium transport needle and attach syringe to the other side port luer (C or D) fitting after you spray with alcohol and wipe. You will have both side ports with syringes on.
- Step 5 Displace the pre-culture 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. Open the right end port. 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.

Step 6 Close the slide clamp and repeat with the opposite syringe.

Step 7 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 act as luer caps. This reduces the number of steps required and helps to prevent contamination. Always use fresh syringes for each harvest.

Place the cartridge onto the pump and begin flow at a setting of between 20 and 25 as indicated on the control box.

**Don't forget to open the left slide clamp to allow flow into the ECS or the cells will die.

Change to a volume of 250mL 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. Continue to double the volume of medium with each medium glucose depletion until the volume reaches 1 liter (2-4 liters for the larger cartridges). Some hybridomas will not tolerate a 1 liter volume; this is indicated by a drop in the glucose rate when switching to a 1 liter bottle. In this case it will be necessary to leave the medium volume at 500 mls and change the medium on a daily basis.



FiberCell 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.

*Medium replacement will vary depending on cells but typically cell growth occurs enough for medium change within the first 3-7 days..

For a more detailed description of the above, and running your FiberCell™ Hollow Fiber Bioreactor, please refer to the user guide. Please refer to harvesting guide for detailed instructions.

4) Harvesting and Maintenance

(Please view the “Low Glucose Rate Harvest” and “High Glucose Rate Harvest” videos on the video CD.)

Harvesting from the hollow fiber bioreactor accomplishes two things. First this is how you obtain your protein of interest. Secondly, harvesting provides a way to control the cell mass inside the cartridge. It is important to keep the glucose rate below 2 grams per day (10 grams per day for the larger cartridges). If the cell mass gets too high the cells will outstrip the ability of the system to deliver oxygen, anaerobic metabolism will result, and production will drop drastically. A surefire way to determine if anaerobic metabolism is occurring is to measure both glucose and lactate. During perfectly aerobic metabolism for every Mole of glucose consumed, one Mole of lactate will be produced. As anaerobic metabolism increases this 1:1 ratio shifts to 1:2 as glucose is consumed more in-efficiently. Since many labs do not have access to lactate measurement the best thing to do is to control the cell mass, keep the glucose consumption at 2 grams per day or less. Once the cells have overgrown the cartridge it can be difficult to reduce the cell mass to acceptable levels. Do not be concerned about cell pellets of 1-5 mls (10-30 mls for the larger cartridges) and lower viabilities. Viability may seem low as you are preferentially harvesting out the non-viable cells. This does not reflect upon the total cell viability inside the cartridge.

A combination of low glucose rate harvesting (to produce the most concentrated product) and high glucose rate harvesting (to reduce cell mass and keep open the pores of the fiber) is optimum. The high glucose rate harvest should be performed once every week or two regardless of the glucose rate in order to help keep the pores of the fiber open.

With the 5kd MWCO cartridges it may not be possible to pull 20-60 mls of medium from the ECS. In this case removal of only 3-5 mls will provide good results.

After about 7-10 days the volume of media in the reservoir bottle should have gone from 125mls to 250 mls to 500 mls to 1 liter. Once you have consumed fully (defined as 50% glucose depletion or around 2 grams per liter of glucose or so) you are ready to harvest. Below are two different harvesting methods. Choose the method that matches your glucose consumption rate keeping in mind that a combination of the two will be appropriate at some time.

Low Glucose Rate Harvest

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

EQUIPMENT AND MATERIALS

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

PROCEDURE

- Step 1 Close the left end port (A) slide clamp and the right end port (B) slide clamp.
- Step 2 Remove one syringe. Open up the left side port (C) and tilt the cartridge up. Pull media out of the ECS. This represents the harvest.
- Step 3 Fill new syringe with fresh media, attach to cartridge and refill the ECS with the fresh media.
- Step 4 Before putting back into the incubator, be sure that both end ports (A and B) are open and that both side ports (C and D) are closed.

High Glucose Rate Harvest

Use this procedure when the glucose rate is 1000mg per day or above. The cell mass needs to be controlled and cell numbers reduced.

PROCEDURE

- Step 1 Close the left end port (A) and open media bottle slightly.
- Step 2 Starting with either side port (C or D), open one port and leave the other side port closed.
- Step 3 Pull out 5-10mLs of cell supernatant into first syringe. The media is being pulled out of the reservoir bottle, through the fibers, and into the syringe. Cells are being pulled out also.
- Step 4 Close first side clamp and repeat for other side port.
- Step 5 When there is an equal amount of media in each syringe, close the right end port (B) open both side ports, (C) and (D) and swish back and forth vigorously a few times to help dislodge cells.

- Step 6 Leave everything in one syringe. This is your harvest. Remember that this harvest is diluted by a factor of two.
- Step 7 Fill new syringes with fresh media, attach to cartridge and refill the ECS with the fresh media.
- Step 8 Before putting back into the incubator, be sure that both end ports (A and B) are open and that both side ports (C and D) are closed.

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