Cartridge Product Insert

C2011
C2008
C2018
C2003
C5011

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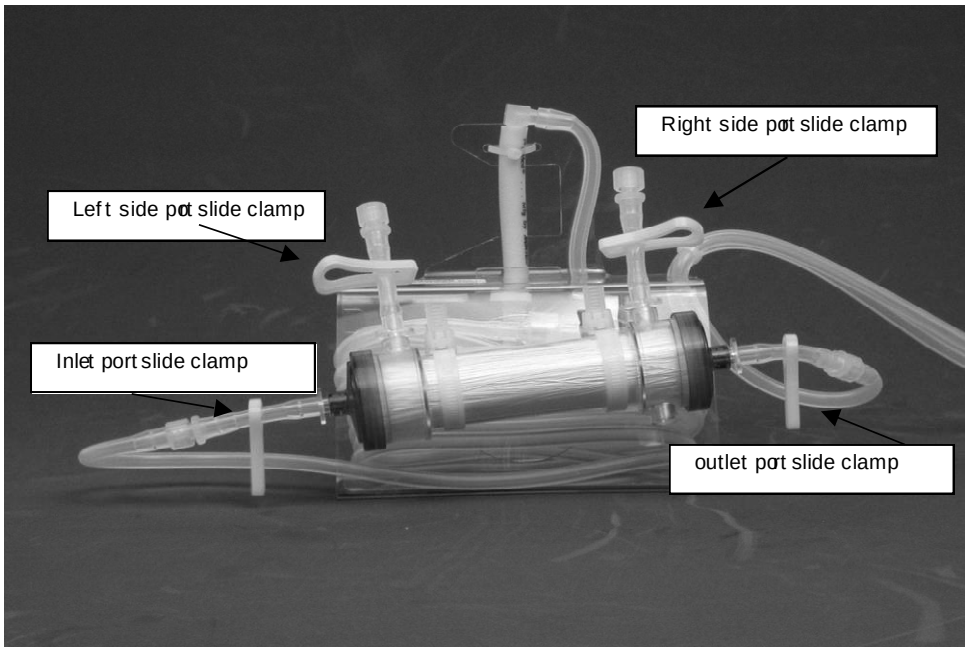

Photo: FiberCell Systems cartridge with ports identified.

Introduction

Read the entire FiberCell Systems User's Manual before using your hollow fiber system. This document provides important information on system set-up, maintenance, and daily monitoring of hollow fiber cultures.

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

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 it necessary to open the hood front be sure to allow time for the air inside the hood to completely exchange.

Module

- Be sure to pre-culture the module for at least 3 days with three changes of medium prior to cell inoculation. For adherent cells pre-culture the module with 2 changes of PBS prior to pre-culture with medium. Change the medium in the extra-capillary space (ECS) at least once.

Cells

- FiberCell Systems Hydrophilic Polysulfone fibers are appropriate for the culture of both adherent and suspension cell lines. Please note the instructions for pre-culturing the module with PBS prior to the inoculation of adherent 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. If serum free medium is desired, perform the adaptation after the cells have reached high density inside the hollow fiber module. Follow the protocol in the FiberCell Systems User's Manual.

- 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 for culture
- Cell culture medium
- FiberCell Systems culture module
- FiberCell Systems reservoir cap, autoclaved
- 10cc or 12cc sterile syringes (luer-lock) (60mls for larger cartridges)
- Alcohol pads
- Spray bottle containing ethanol
- Large bore needles
- 50ml conical centrifuge tubes
- Lactate test kit (cat# 735-10, Sigma Chemicals)
- Lactate standards (cat# 735-11, Sigma Chemicals)

Module Set-Up

Prior to inoculating cells into the FiberCell Systems module the following steps must be carried out. Perform all steps inside a clean laminar flow hood.

1) **Sterilize** the reservoir cap/bottle assembly
2) **Connect** the reservoir cap/bottle to the flow path
3) **Pre-culture** the hollow fiber module with complete cell culture medium

Sterilization of the Reservoir Cap/Bottle Assembly

Two sizes of reservoir cap/bottle assemblies are available. The 33mm cap (catalog # A1005) is designed to fit standard glass cell culture media bottles. The 38mm cap (catalog # A-1006) is designed to fit standard plastic cell culture media bottles. The materials of the cap and the included glass bottle are designed to withstand repeated autoclave cycles.

1) Insert the reservoir cap into the bottle. Screw the cap so as to have a loose fit. The cap must be loose enough to allow steam to penetrate.
2) Attach fresh reservoir cap tubing (non-sterile, included with each new cartridge) to the cap.
3) Loosely wrap the luer fittings on the end of the reservoir cap tubing with foil or autoclave paper.
4) Place entire assembly into an autoclave bag and seal.

5) Steam autoclave at 121°C. Use a dry cycle if possible. Wet autoclave paper is not a sterile barrier.
6) Remove from autoclave and allow cooling prior to use.

Connection of the Reservoir Cap to the Flow Path

The FiberCell Systems culture module is supplied pre-sterilized. The two luer connectors on the flow path should be connected to the two luer fittings on the reservoir cap. There is no directionality; it makes no difference which flow path tubing is connected to which luer fitting on the reservoir cap.

1) Remove FiberCell module from it's packaging. Check the luer fittings between the hollow fiber module and the flow path and insure that they are finger tight. These luer fittings are at each end of the cartridge.
1) Remove the foil from one of the luer fittings on the reservoir cap
2) Choose one of the luer fittings on the flow path. Spray with ethanol and wipe with an alcohol pad. Remember that it is not the application of ethanol that sterilizes, it is the evaporation. Allow time for evaporation to occur.
3) Remove the luer cap from the luer fitting and attach luer fitting to the reservoir cap. Apply 1/2 turn counter rotation to the tubing prior to attachment to prevent kinking of the tubing.
4) Repeat steps 3 and 4 to attach the other luer fitting.
5) Check fittings and be sure that they are attached tightly.

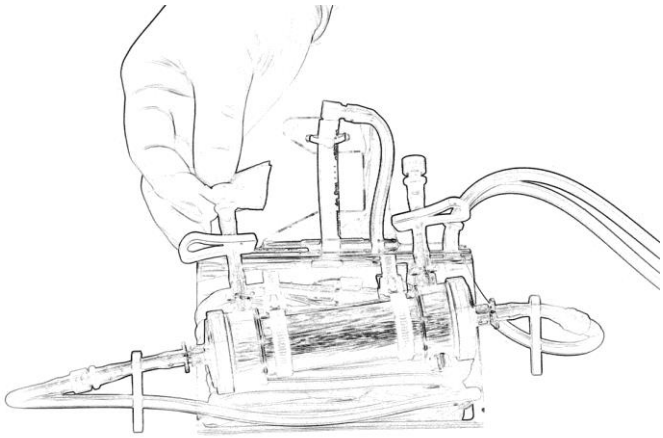

Figure 2 Spray luer fittings with 70% ethanol and wipe with alcohol pad prior to connecting syringes or attaching fittings.

Pre-culture

The FiberCell Systems module should be pre-cultured for a minimum of three days with three changes of medium, 24 hours each. If working with anchorage dependant cell lines two steps of pre-culturing with sterile PBS should be performed. This ensures that the cartridge is completely equilibrated with the cell culture medium and removes any wetting agent that might be present on the fiber. Bubbles are removed during the pre-culture step. The pre-culture period is also used to check for any leaks and to ensure cartridge sterility.

Important! If working with adherent cell types two steps of pre-culture using sterile PBS should be performed prior to filling the cartridge with cell culture medium. These should consist of a volume of 125mls of PBS for a minimum of 24 hours each. Simply follow the directions below for filling the cartridge with cell culture medium. After the two changes of PBS begin pre-culture using cell culture medium as instructed below.

Priming the flow path and cartridge

- At least 100mls of medium should be used for pre-culture. The flow path and cartridge for the medium sized cartridges hold about 30 mls of medium. The flow path and larger cartridge hold about 80 mls of medium.
- The same formulation of medium used for culture should be used for the pre-culture step. Serum, cytokines and/or other expensive cell culture medium components may be added at third and final step.

Fill the flow path with medium

1) Fill the empty reservoir bottle with 100ml culture medium. Do not pour. Use a 25ml or 50ml pipette. Leave the reservoir cap loose by 1/2 turn.
2) Using thumb and forefinger manually pump the medium until all air is pumped out of the flow path.

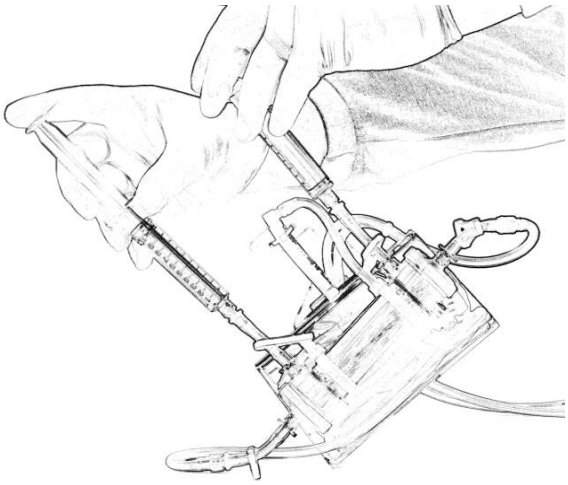

Figure 3 Filling the ECS with medium. Left and right end ports closed. Left and right side ports open.

Fill the extra-capillary space with medium

1) Close both end port slide clamps. Insure that both side port slide clamps are open.
2) Place 25mls of medium into a 50ml conical centrifuge tube. Using a syringe needle fill a 10ml (or 60ml for the larger cartridges) syringe with medium.
3) Spray ethanol around the side port luer fitting and wipe with an alcohol pad. Carefully remove luer cap with alcohol pad and set aside aseptically. Luer cap can be re-used if handled carefully or they may be autoclaved and re-used.
4) Attach medium-filled syringe to side port luer.
5) Following guidelines above attach empty syringe to other side port.
6) Tilt cartridge upwards and fill with medium taking care to remove all air present in the ECS. If volume of medium is not sufficient, repeat.
7) Aseptically replace luer caps. If desired syringes may be left connected to the cartridge and replaced each time medium is changed, cells are loaded or secreted product is harvested.
8) Close side port slide clamps and open end port slide clamps
9) Connect the FiberCell hollow fiber module to the FiberCell pump unit. Set at medium flow rate.

Pre-culture should be allowed to take place for a minimum of three days with the cell culture medium in the reservoir bottle changed at least three times. The medium in the ECS should be changed at least once by following the harvest protocol outlined below.

Inoculating Cells into the ECS

Use rapidly dividing cells with at least 90% viability. Minimize the time between harvesting the cells from flasks and inoculation into the cartridge. Do not store cells on ice prior to inoculation. Use the medium from the harvested cells if possible. Do not pin the cells down and re-suspend in fresh medium. If cells require concentration spin them down and re-suspend in the medium they were harvested in. Simply spin down and decant off excess medium and re-suspend in the same medium. A minimum of 5 X 107 hybridoma cells should be loaded into the medium sized cartridge (cat# C2011), 1-2 X 108 hybridoma cells should be loaded into the larger cartridge (cat# C2018).

Cell Inoculation Numbers

Cartridge	Cell Type	Cell Number
C2011	Hybridoma	5 X 107
C2011 , C2008	Suspension	1-2 X 108
C2011, C2008	Adherent	Equal to 50% confluence of 2,200cm2
C2018	Hybridoma	1-2 X 108
C2018, C2003	Suspension	5 X 108
C2018, C2003	Adherent	Equal to 50% confluence of 1.2m2

1. Close the left and right end-port slide clamps
2. Draw cells to be inoculated from 50ml conical centrifuge tube into a sterile 10ml syringe (60ml for larger cartridges) using sterile large gauge needle.
3. Spray the left side port luer connection with 70% ethanol and wipe with an alcohol pad. Carefully remove the luer plug or syringe.
4. Place syringe loaded with cells onto the side-port luer.
5. Following same aseptic procedure attach an empty syringe to the right side-port luer fitting.
6. If closed, open the side-port slide clamps
7. Gently flush the cell suspension through the cartridge ECS back and forth between the two syringes 3-5 times. This insures even distribution of the cells.
8. After the final flush leave 50% of the volume of cells in each syringe.
9. Loosen the reservoir cap by 1/2 turn.
10. Leave the left end port slide clamp closed. Open the right hand end port slide clamp. This will allow excess medium to flow into the reservoir bottle. Cells cannot pass across the fiber and will remain trapped in the ECS.
11. Close the right side port slide clamp.
12. Gently depress the plunger of the syringe attached to the left side port until all of the medium and cells have been forced into the ECS.
13. Close the left side port slide clamp and open the right side port slide clamp.

14. Gently depress the plunger of the syringe attached to the right side port until all of the medium and cells have been forced into the ECS.
15. Close the side port slide clamps. You may leave the syringes attached or replace with sterile luer caps using aseptic technique.
16. Tighten the reservoir cap
17. Open **the left end port slide clamp!** This step is very important. If the left end port slide clamp is left closed no cell culture medium can circulate and the cells will die within 2 hours.
18. Place the cell culture module into the FiberCell pump inside a CO2 incubator and set flow rate to a medium level (20-30mls/min) After 3 days increase flow rate to maximum.

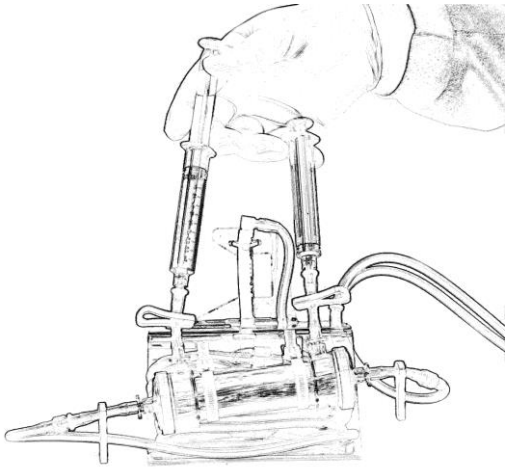

Figure 4 Inoculating cells. Left and right end ports closed. When attaching syringes left and right side ports closed. After syringes attached open side ports and flush medium containing cells back and forth through the ECS 3-4 times to insure good mixing. Leave 50% of the cell inoculum in each syringe.

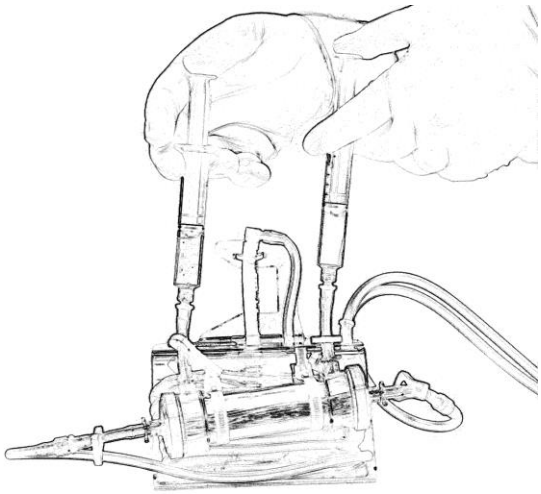

Figure 5 Inoculating cells. Left end port closed. Right end port open. Loosen reservoir cap 1/2 turn. Close right side port (left side port open) and gently push cells and medium from syringe into ECS. Cells will remain trapped in ECS and excess medium will flow into reservoir bottle. Close right side port and open left side port. Gently push cells and medium into ECS. Open left end port slide clamp. When finished, tighten reservoir bottle cap. **Check to make sure that the left and right end port slide clamps are open!**

Harvesting of Cells and Product from the ECS

Care should be taken to not remove too many cells from the cartridge during the harvest process early in cartridge life. When the lactate rate is less than 1,000mgs/day (for C2011 and C2008) or less than 4,000 mgs/day (for C2018 and C2003) use a single harvest by draining the ECS only once. When lactate level is higher than the above, repeat the harvest steps once or twice more in order to remove cells. Medium can be flushed back and forth between the syringes to remove more cells.

Begin harvesting after first full liter (or two liters for C2003 and 2018) of medium has been consumed.

Harvest hybridomas every two days, harvest recombinant proteins every day.

1. Close the left and right end port slide clamps
2. Spray the left side port luer fitting with 70% ethanol and wipe with an alcohol pad.
3. Attach an empty sterile syringe (10mls for the medium cartridge, 60mls for the large cartridge) to left side port luer fitting.

4. Using same aseptic technique described above attach an empty syringe filled with sterile air to the right side port luer fitting.
5. Open the left and right side port slide clamps. (side port slide clamps should always be closed while the hollow fiber module is attached to the pump system)
6. Tilt the hollow fiber module to a near vertical position with the empty syringe at the bottom and the air filled syringe at the top.
7. Withdraw the contents of the ECS into the bottom syringe.
8. Remove syringe containing the harvested ECS and transfer the harvested material to an appropriate container such as a 50ml conical centrifuge tube.
9. Fill a fresh sterile syringe with fresh medium (using a large gauge needle) and attach to the empty sideport. Refill the ECS with fresh medium.
10. Either replace the syringe onto the side port luer fitting or aseptically place a sterile
11. luer cap onto the side port luer fitting.
12. Close the left and right side port slide clamps
13. Open the left and right end port slide clamps.

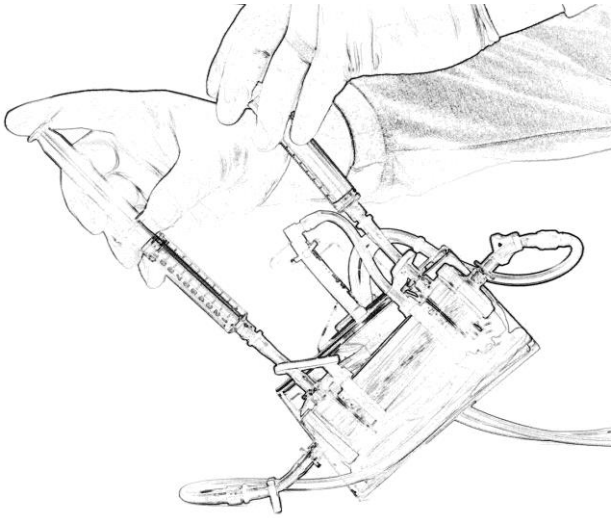

Figure 6 Harvesting product. Close left and right end port slide clamps. Attach syringes to side ports. If lactate rate is too high remove cells by flushing back forth between the syringes. After harvest is complete be sure to open left and right end port slide clamps.

Before placing module into pump system check to be sure that the left and right end port slide clamps are fully open. Failure to open the end port slide clamps will result in loss of cell viability within 2 hours.

FiberCell Daily Maintenance Schedule

(volumes in parentheses refer to C2003 and C2018 cartridges)

Day	**Procedure**
0	Inoculate Cells
1	25mls media (250mls media)
1	Check lactate Level
2-3	Check lactate, replace medium with 250mls (500mls) when greater than 1mg/ml
4-5	Check lactate, replace medium with 500mls (1 liter) when greater than 1 mg/ml
5-7	Check lactate, replace medium with 1,000mls (2 liters) when greater than 1.5 mg/ml
8-10	Check lactate, replace medium when greater than 1.5 mg/ml. Harvest Antibody from ECS every other day. Harvest proteins every day
11+	Check Lactate, determine lactate trigger point, harvest antibody every two days (proteins every day) , change medium at trigger point.

Notes: CHO cells and 293 cell lines can generally tolerate higher levels of lactate than hybridomas. 2 liter and 4 liter reservoir bottles with 38mm neck are available from Nalgene.

2 liter: catalog number DS2205-0210
4 liter: catalog number DS2205-0010

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