

HOLLOW FIBER BIOREACTORS AND RELATED PRODUCTS

Designed for the following applications:

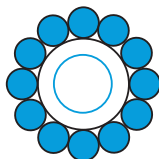
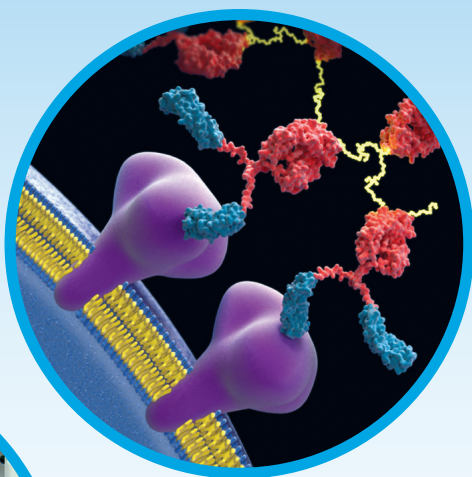
Endothelial Cell Culture

In Vitro Toxicology

Monoclonal Antibody Production

Recombinant Protein Production

Secretome and Conditioned Medium Production



FiberCell Systems Inc.
a better way to grow cells

The FiberCell® System: Hollow Fiber Cell Culture

A fundamentally different approach to cell culture is now available. Modeled after the mammalian circulatory system hollow fiber cell culture offers the most *in-vivo* like manner to grow cells in any laboratory.

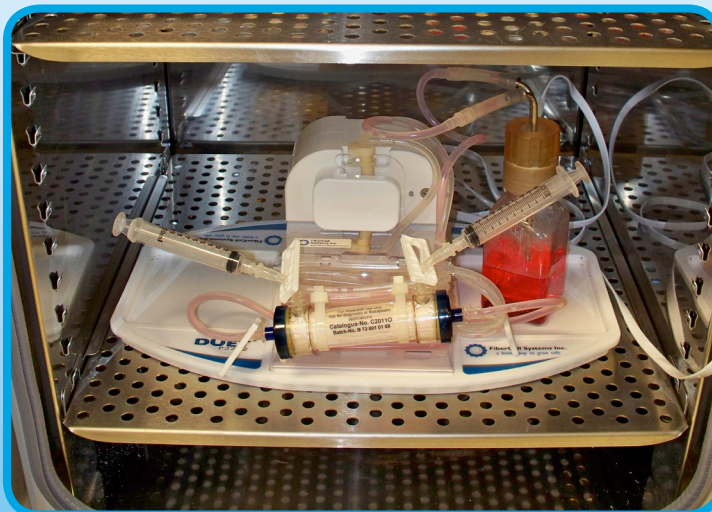
Hollow fibers are small filters shaped like drinking straws approximately 200 microns in diameter whose molecular weight cut-off can be between 5kd and .1 microns. These fibers are sealed into a cartridge housing so that medium pumped through the end of the cartridge will flow through the inside of the fibers while the cells are grown on the outside of the fibers. These fibers create a defined semi-permeable barrier between where the cells are growing and the medium is flowing. Since the cells are attached to a porous support splitting of the cells is not required and cultures can be maintained for many months of continuous production. When secreted products are retained in the small volume of the extra-capillary space their concentration can be 100X or more than that of flask or roller bottle culture.

Hollow fibers also provide a tremendous amount of surface area in a small volume. Cells can grow at densities of greater than 1×10^8 /ml or more, *in vivo* like cell densities. Cell culture at high densities has the following benefits:

- Reduces serum requirements
- Facilitates adaptation to serum free medium including CDM HD
- Increases the concentration of secreted products by 10 to 100 times
- The most *in vivo* like way to grow cells



The FiberCell® Systems Duet pump and cartridges are designed to be a simple but effective means for bringing the benefits of hollow fiber cell culture to any laboratory. The Duet pump fits into a standard CO₂ incubator, which provides temperature, and gas control. Cartridges are easily removed from the Duet to work with in the laminar flow hood.



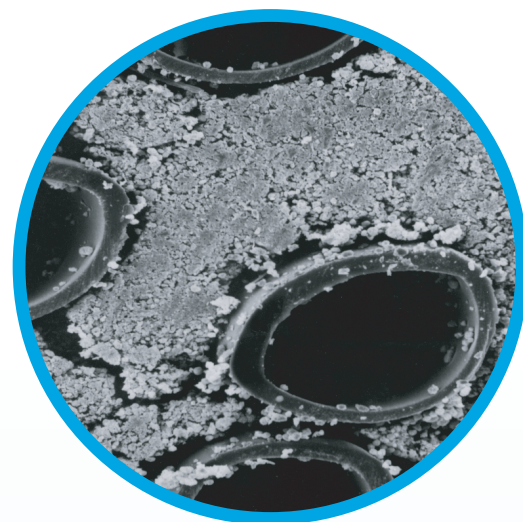
The FiberCell® Systems Duet pump provides flexible flow rate support for all of the FiberCell® Systems hollow fiber bioreactor cartridges. Continuous flow is required to provide for nutrition support and constant oxygenation of the culture modules. The Duet pump utilizes a unique positive pressure displacement pumping action on the pump tubing of the flow path. Squeezing of the tubing in conjunction with the two one-way check valves produces a frictionless pumping action that prevents the generation of particles that can clog the fibers. This frictionless mechanism is the reason that FiberCell® Systems modules have supported continuous culture of cells up to 2 years of continuous culture

The FiberCell® Systems Duet pump can generate from 5 ml to 140 ml per minute flow rate depending upon the cartridge used. The loop of gas permeable silicone tubing provides gas exchange while maintaining the system in a closed, biosafe manner.

FiberCell® Systems Hollow Fiber cell culture cartridges are the method of choice for the production of 10mg to gram quantities of recombinant proteins and monoclonal antibodies and the culture of 10^9 to 10^{11} cells.

What is the FiberCell® System advantage?

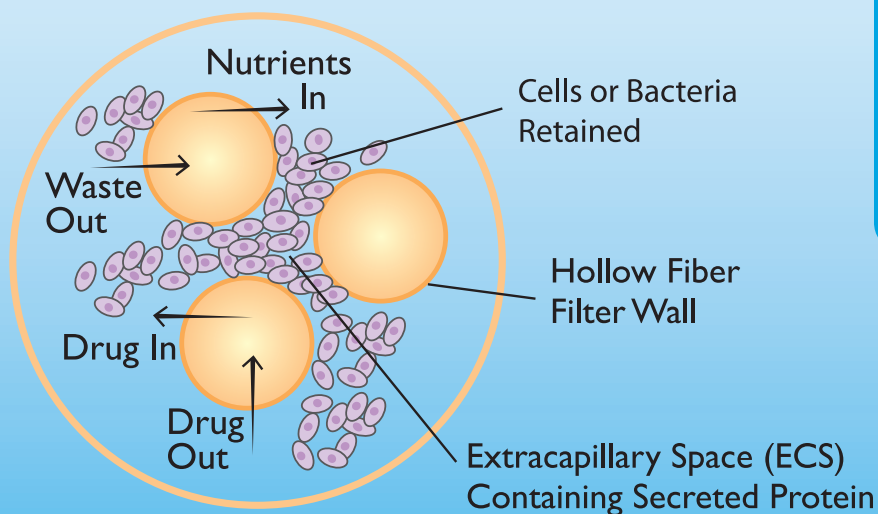
- Concentrates secreted proteins and antibodies up to 100X or more in the small volume of the extra-capillary space
- Eliminates splitting because cells are bound to a porous support; culture can last many months or longer
- Improves cell viability and reduces apoptosis
- Improves cell functions such as protein folding and post-translational modifications, creating homeostatic, more *in vivo* like culture conditions
- Dialyzes waste and inhibitory cytokines away from the cells
- Enhances bio-safety with a closed system
- Handles a large numbers of cells (up to 10^{11})
- Eliminates dead spots with uniform fiber spacing and "wavy" fibers within the cartridge, optimized for both suspension and adherent cells
- Provides flexible control options with unique variable flow rate pump system
- Ensures long cartridge life and facilitates nutrient and waste exchange cross the fiber due to positive-pressure displacement pump system



ABOVE: Cross-section of hollow fibers in FiberCell® Systems hollow fiber bioreactor. High-density cell growth is shown in extracapillary space.

- Cells grow on and around hollow fibers.
- Fiber geometry is optimized for both adherent and suspension cell types.
- Small molecules such as lactate and glucose can easily cross the fiber.
- Large molecules such as monoclonal antibodies and proteins are retained and concentrated in the small volume of the extra capillary space.

The FiberCell® System



A single FiberCell® Systems hollow fiber cartridge is equivalent to harvesting 20-200 roller bottles daily.



SUGGESTED APPLICATIONS

MONOCLONAL ANTIBODY PRODUCTION

A hollow fiber bioreactor from FiberCell® Systems is the ideal method for the *in vitro* production of 25mg on up to gram quantities of a monoclonal antibody. A technologically advanced high-gross-filtration rate hydrophilic polysulfone fiber with a molecular weight cut off (MWCO) of 20kd is specified. Hybridomas secrete an inhibitory factor called TGF-Beta that in its active form has a molecular weight of 27kd. The MWCO of the hollow fibers permits this inhibitory cytokine to diffuse away while the secreted antibody and cells remain in the small volume of the extra-capillary space. The gross filtration rate of the polysulfone fiber is 10X higher than that of cellulosic fibers for more rapid exchange of nutrients and waste products. Cell densities of 10^8 or higher are attained facilitating the reduction of serum, the adaptation to commercially available serum free formulations and the use of FiberCell® Systems' CDM-HD (pg 7.)

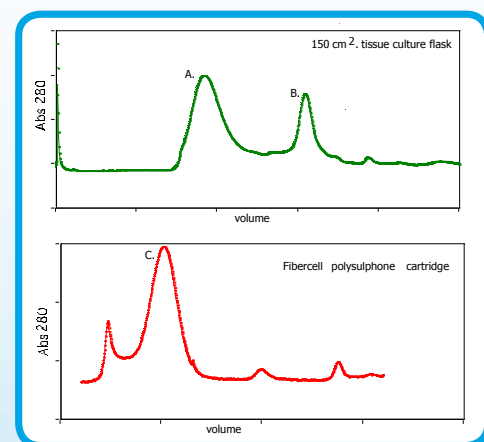
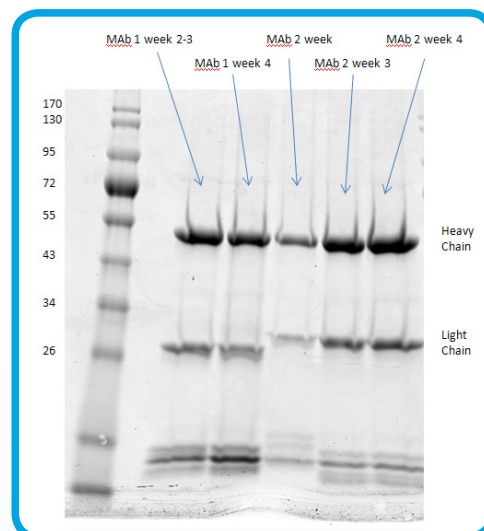
SECRETED PROTEIN PRODUCTION

The production of recombinant proteins and conditioned medium from mammalian cells can be such a cumbersome process that for many laboratories the use of mammalian expression systems is to be avoided. Large numbers of plates, flasks or roller bottles are required or the use of a stirred tank vessel is mandated. The cell culture conditions provided by these methods are inherently non-physiologic. Hollow fiber bioreactors provide a tremendous amount of surface area for cell attachment and the high cell density permits the use of our CDM-HD serum replacement and can also result in improved protein folding and more uniform post-translation modifications. Protein concentrations are in the range of 100 ug to 300 ug/mL/day and the production of 10's of mg on up to gram quantities of proteins is possible. The protein of interest becomes a significant component of the harvested supernatant and the small volumes facilitate purifications.

SECRETOME AND CONDITIONED MEDIUM PRODUCTION

FiberCell® Systems hollow fiber cell culture cartridges are ideal for the production of conditioned medium and generating the secretome from various cell types including tumor cell lines. Large numbers of cells can be supported for several months of culture and can be equivalent to 200-400 roller bottles. Proper selection of the MWCO will provide useful information as to the size of the conditioning factors of interest. Recent studies have shown that apoptosis is significantly reduced in the hollow fiber environment. The supernatant is free of intracellular proteins that might come from cell lysis. When used in conjunction with CDM-HD it can be a convenient and powerful tool for producing conditioned medium and secretomes free from interfering serum and lytic proteins.

- Conditioning factors will be 10-100X higher in concentration
- Facilitates initial determination of molecular weights
- Free from interfering proteins
- Can generate the equivalent of many liters of supernatant.



Comparison of a purified recombinant human hexameric IgG produced from CHO cells grown in either a 150cm² tissue culture flask (upper panel) or a FiberCell polysulfone cartridge (lower panel). Gel filtration chromatography reveals incomplete polymerization of the hexameric IgG produced in the flask. Both a highly polymerized hexameric IgG (peak A.) and non-polymerized (peak B.) are observed. In contrast, the same protein produced in a FiberCell cartridge is expressed almost entirely in highly polymerized form (peak C.) 478 mg of purified protein were produced in 2 months in a volume of less than 5 liters using catalog #2018.

Data courtesy of Dr. Jim Arthos - Bethesda, MD.

IN VITRO TOXICOLOGY

Hollow fiber cartridge systems from FiberCell® Systems have been used to model pharmacologic bio-availability for different antimicrobial drugs and to mimic dosage profiles that generate resistant organisms. This system mimics clinically relevant drug dosage profiles in a rigorously controlled *in vitro* system composed of a hollow fiber cartridge and associated pumps and reservoirs. It is superior to a one-compartment model as bacterial numbers are preserved and the closed nature of the system allows the study of pathological organisms in a safe and controlled manner. It is superior to animal models in that dosage and elimination profiles can be modeled on human kinetics and permits the use of organisms not supported by animals. The small volume of the organism compartment permits rapid and precise changes in drug concentration. Hollow fiber technology offers higher levels of reproducible control to complex growth, infection, treatment, and sampling regimens. The *in vitro* hollow fiber model can reduce the amount of animal studies required and can generate clinically relevant data that can be used in conjunction with human studies and is recognized by regulatory agencies. The advantages of using the FiberCell® Systems hollow fiber bioreactor are:

- Mimics *in vivo* drug dosage profiling
- Any dosage can be easily evaluated-extreme doses can be modeled
- Closed Biosafe System
- Well tested and accepted protocols. Antibiotics, anti-virals, anti-cancer agents, and anti-fungals have all been tested in the FiberCell® Systems.
- Dosage and metabolic profiles can be more precisely controlled.
- Different fiber chemistries can be optimized for particular drugs.

ENDOTHELIAL CELL CULTURE

The FiberCell® Systems PS+ module (cat#4300-C2025) is especially designed for the culture and study of endothelial cells under flow. The PS+ fiber can easily be coated with extra-cellular matrix proteins (and/or cytokines and antibodies). This allows the study of endothelial cells in a more physiologic environment when compared to other methods. As a result, typical cell morphology is retained, a monolayer is formed and tight junctions are created. These culture conditions more closely mimic *in vivo* cell growth.

The microporous nature of the fibers and the ability to control the extra-cellular matrix provides an ideal system for cellular co-cultivation with other cell types.

- Inner surface area of the fibers is equal to a T75 flask
- Shear stress of .5 dynes/cm² to 25 dynes/cm² can be generated
- Approximately 100 micrograms of RNA can be isolated from each cartridge
- Most types of microscopy can be performed including immunohisto-chemical techniques

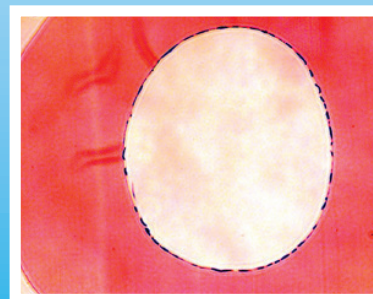
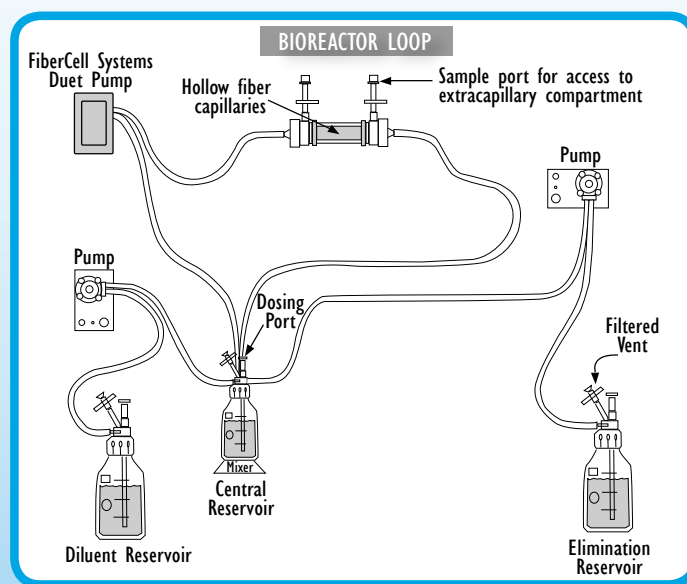


Figure 7 Bovine Aortic endothelial cells grown on a matrix of fibronectin.

THE FIBERCELL® SYSTEMS DUET

Duet Pump

The FiberCell® Systems Duet pump provides flexible flow rate support for up to two of the FiberCell® Systems hollow fiber bioreactor cartridges with totally independent medium reservoirs. Continuous flow is required to provide for nutrition support and constant oxygenation of the culture modules. The Duet pump utilizes a unique positive pressure displacement pumping action on the pump tubing of the flow path. Squeezing of the tubing in conjunction with the two one-way check valves produces a frictionless pumping action. Peristaltic pumps rub on the tubing causing pieces of the tubing to break off and move into circulation where they can cause blockage of the fibers. This frictionless mechanism is the reason that FiberCell® Systems modules have supported continuous culture of cells up to 2 years of continuous culture.



FiberCell® Duet Specifications

Dimensions	9.5 x 16.5 x 8" (w x l x h)
Weight	9.5 lbs
Voltage	100, 120, 220 (50 or 60 hz)
Flow Rate	1-160mls/minute, continuously variable

P3202 FiberCell Duet Pump, Universal Voltage, 100V, 120V, 220V

- Occupies 1/2 shelf, 1/3 height of a standard CO2 incubator
- Separate medium reservoirs eliminate cross contamination
- Duet can support all FiberCell hollow fiber cartridges
- High flow rate for optimum culture using large cartridges
- 10 ft flat power cord is designed to fit in the incubator door when closed
- Magnetic strips for controller attachment provided with unit, for ease of controller placement
- Full Two year limited warranty

Constant flow rate and Oxygenation are imperative to the support of hollow fiber cell culture modules. The FiberCell® Systems Duet pump system can generate from 1ml to 140 mls per minute of flow rate depending upon the cartridge used. Silicone tubing is gas permeable and can be used to provide for gas exchange while maintaining the system in a closed, biosafe manner. The loop of silicone tubing that is part of the cartridge flow path stand provides the gas exchange for the system. The larger cartridges (C2003 and C2018) along with the C5011 contain twice the amount of tubing as the medium sized cartridges and also have a larger pump tubing to provide for increased flow rate increasing the oxygenation capacity of these systems.

RESERVOIR CAPS

Reservoir Cap

The FiberCell® Systems Reservoir Cap is a re-usable, autoclavable cap designed to fit onto a standard cell culture media bottle. The 33mm cap (cat# A1005) will fit onto standard glass medium bottle and the 38mm cap (cat# A1006 will fit onto standard plastic medium bottles.

The 45 mm cap fits large mouth cell culture media bottles.



A1005	33mm Reservoir Cap Assy
A1006	38mm Reservoir Cap Assy
A1007	In Vitro Toxicology Cap
A1008	Stainless Steel Cap for 45mm neck size

FiberCell® Systems offers additional technical support for our systems as well as for other hollow fiber bioreactor systems.

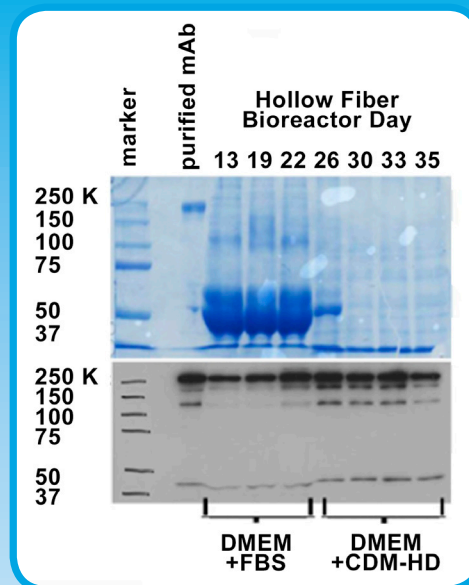
SERUM REPLACEMENT

CDM-HD

The FiberCell® Systems CDM-HD Serum Replacement is a chemically defined, protein free serum replacement that permits any basal medium such as DMEM to be used without serum. CDM-HD is designed specifically for the culture of cells at high density and optimized for use in our hollow fiber bioreactor systems. CDM-HD provides lot-to-lot consistency, simplifies purification and is an economical replacement for serum. It is available as a dry powder to make up one liter and is used at a concentration of 10%.



CDM-HD-I Powder to make 1 Liter



SUGGESTED APPLICATIONS FOR FIBERCELL® SYSTEMS CARTRIDGES

C2025 0.1µm pore size for the highest exchange rates. Activated fiber for attachment of matrix proteins, cytokines and antibodies. Ideal fiber for endothelial cell and hepatocyte culture.

C2008 Low MWCO (5 kd @ 50%) hydrophilic fiber for trapping smaller molecules. Suggested for recombinant proteins between 20 kd and 100 kd. Appropriate for suspension and adherent cell lines including CHO, HeLa and 293. Can support up to 10⁹ cells and produce 1-2 mg of recombinant protein per day.

C2011 High MWCO (20 kd @ 50%) hydrophilic fiber for trapping larger molecules, hybridoma culture and lymphocyte culture. 20 kd MWCO allows TGF beta and TNF alpha to diffuse away while retaining antibody. Can support up to 10⁹ cells and produce 10-50 mg of monoclonal antibody every two days. Suggested for recombinant proteins larger than 100 kd and will produce 1-2 mg of protein per day.

C5011 Same sized cartridge as the C2011 but optimized for maximum monoclonal antibody production by increasing flow rate and doubling the amount of oxygenation tubing and will produce between 20 mg and 100 mg of antibody every two days.

C2003 Low MWCO (5 kd @ 50%) hydrophilic fiber for trapping smaller molecules. Suggested for recombinant proteins between 20 kd - 100 kd. Appropriate for suspension cell lines including CHO, HeLa and 293 cells. Can support up to 5 x 10¹⁰ cells and produce 5-10 mg of recombinant protein per day.

C2018 High MWCO (20 kd @50%, 95% @ 100 kd) for trapping larger molecules in the range of 100 kd and larger such as recombinant proteins from CHO, HEK 293 and other cell types. Can support up to 5 x 10¹⁰ cells and produce 5-10 mg of protein per day.

C3008 utilizes a cellulosic fiber with a nominal MWCO of 5KD. C3008 is intended primarily for use for in vitro toxicology applications where organic based drugs may exhibit non-specific binding to other fiber types.

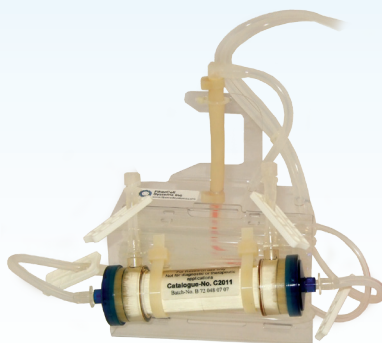
C4005 The C4005 cartridge is intended for use in larger hollow fiber cell culture systems from other manufacturers and does not include a flow path stand or oxygenator tubing.

C4020 The C4020 cartridge is intended for use in larger hollow fiber cell culture systems from other manufacturers and does not include a flow path stand or oxygenator tubing.

Oxy 0.6 The Oxy 0.6 is a replacement oxygenator cartridge for 1.0 m² oxygenators available from other manufacturers. It utilizes a new fiber with better gas transfer and reduced medium weepage.



C2025



C2008
C2011



C2003
C2018

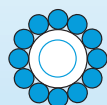
CARTRIDGE SPECIFICATIONS

Stock No.	Size	Surface Area	Fiber Type	Packing Density	ECS Volume	MWCO 50%	MWCO 95%	Maximum Cell#
C2025	Small	75cm ²	Activated PS	30%	1.5mL	0.1µm	0.1µm	10 ⁸
C2008	Medium	3000cm ²	low flux PS	50%	20mL	5kd	20kd	10 ⁹
C2011	Medium	3000cm ²	high flux PS	50%	20mL	20kd	100kd	10 ⁹
C5011	Medium	3000cm ²	high flux PS	50%	15mL	20kd	100kd	2 x 10 ⁹
C2003	Large	1.2m ²	low flux PS	50%	70mL	5kd	20kd	5 x 10 ¹⁰
C2018	Large	1.2m ²	high flux PS	50%	70mL	20kd	100kd	5 x 10 ¹⁰
C3008	Medium	2000cm ²	cellulosic	38%	12mL	5kd	20kd	N/A
C4005	X-Large	2.5m ²	low flux PS	50%	150mL	5kd	20kd	10 ¹¹
C4020	X-Large	2.5m ²	high flux PS	50%	150mL	20kd	100kd	10 ¹¹
Oxy 0.6	12mls	6000cm ²	Hydrophobic polyethylene	N/A	40mL	100kd	10kd	N/A

OUR MISSION

FiberCell® Systems Inc. is committed to providing easy-to-use hollow fiber cell culture systems that allow researchers and biotechnology companies to produce quantities of cells and cell products not possible using standard techniques. FiberCell® Systems Inc. continuously strives to develop new methods and approaches for hollow fiber systems. Personal service and responsive post-sale support is the cornerstone of our business.

TO ORDER, CONTACT:



FiberCell Systems Inc.
a better way to grow cells

FiberCell Systems Inc.

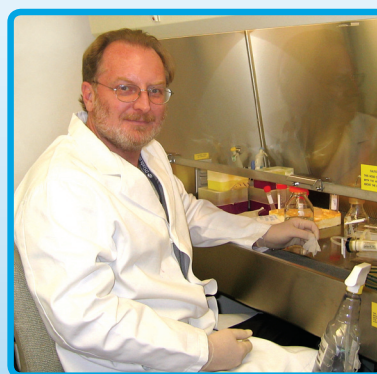
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