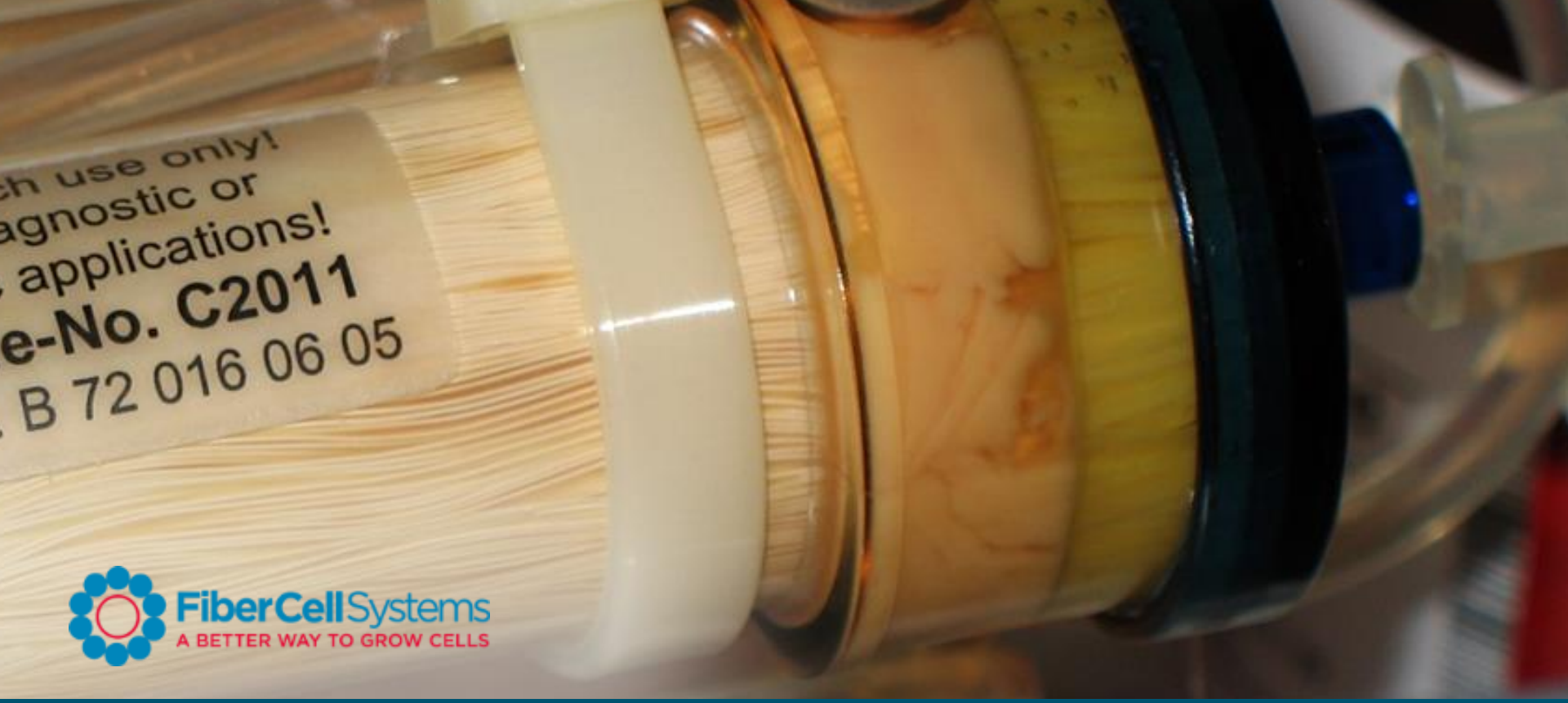
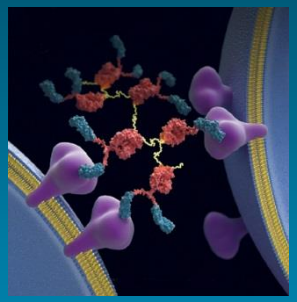


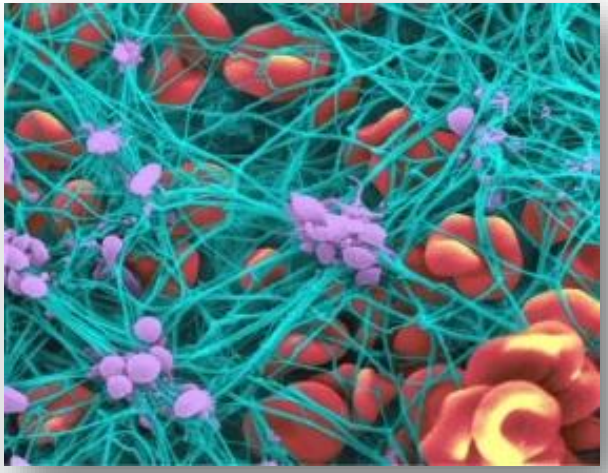
Hollow Fiber Bioreactors and Difficult-to-Express Proteins

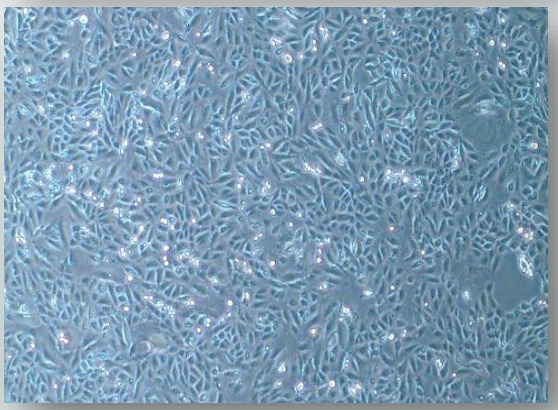


By John J. S. Cadwell

www.fibercellsystems.com

Cell Culture Through the Ages

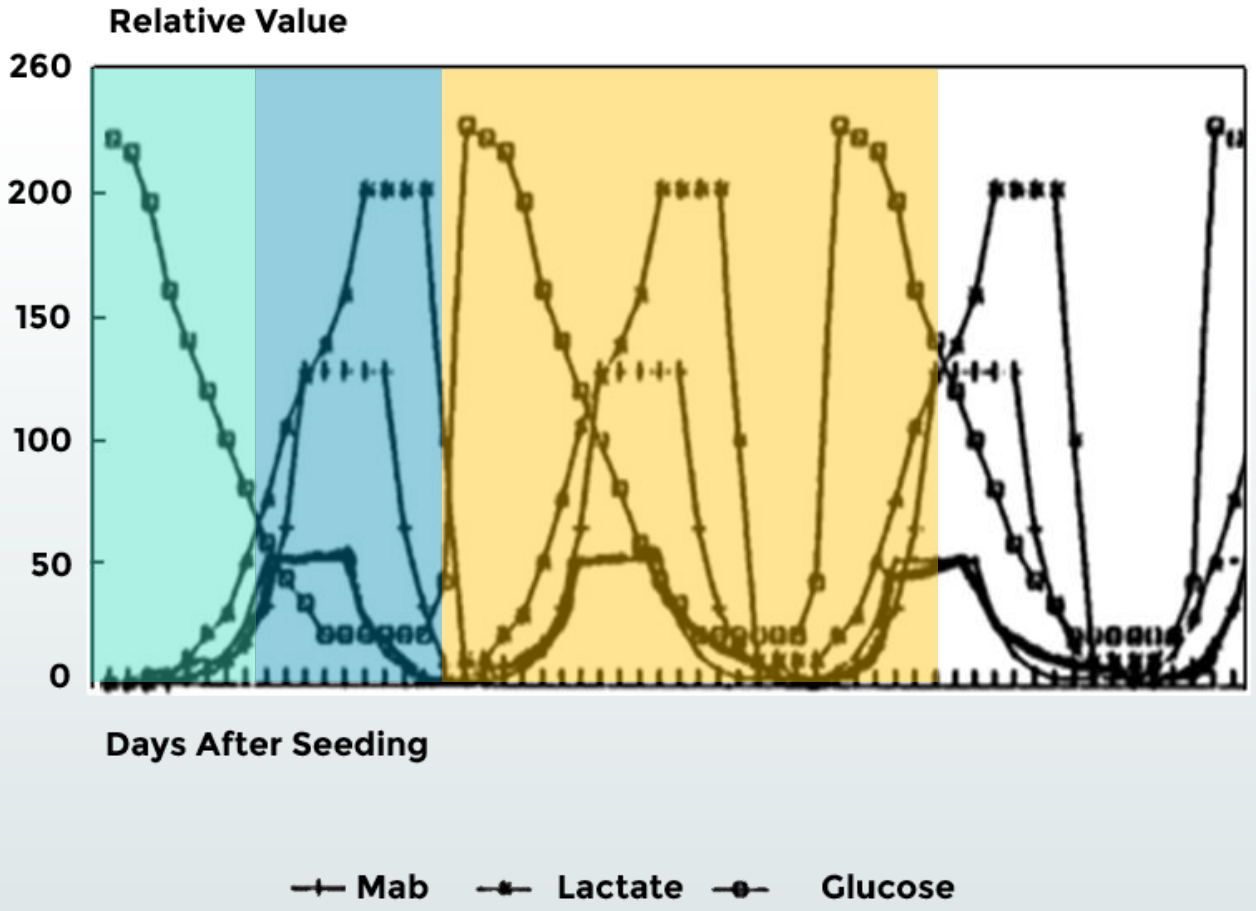




Cell Culture Options for Scale-up

- Roller Bottles
- Cell Factory
- Cell Cube
- Cell Culture Bags
- Spinner Flasks
- Bioreactors

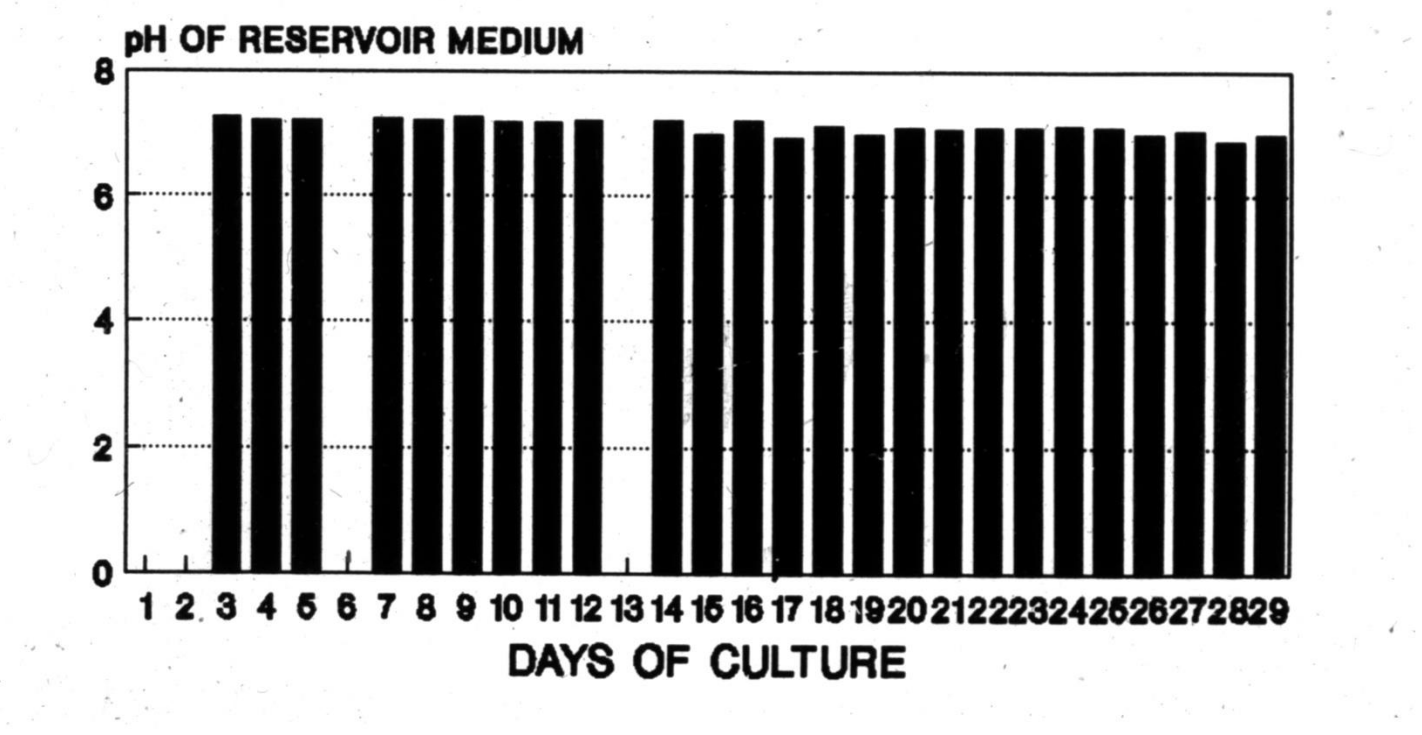
“Feast or Famine”



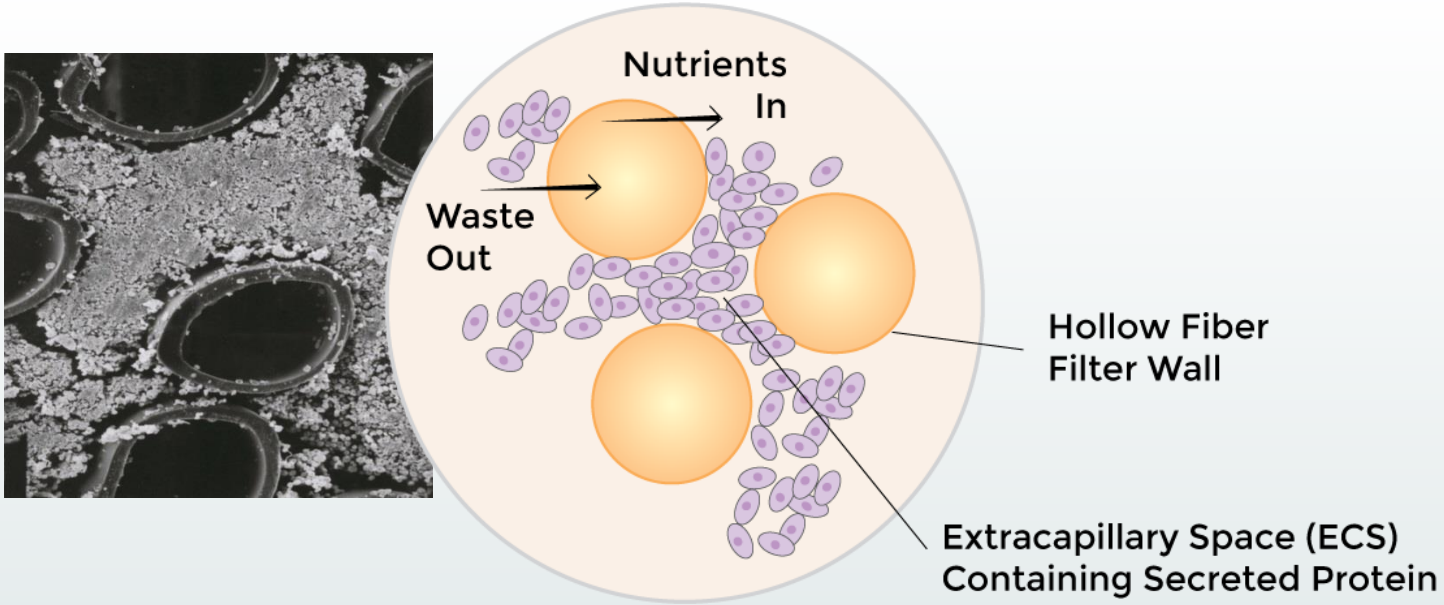
FiberCellSystems
A BETTER WAY TO GROW CELLS

www.fibercellsystems.com

Hollow Fiber Culture of CHO Cells, pH Changes

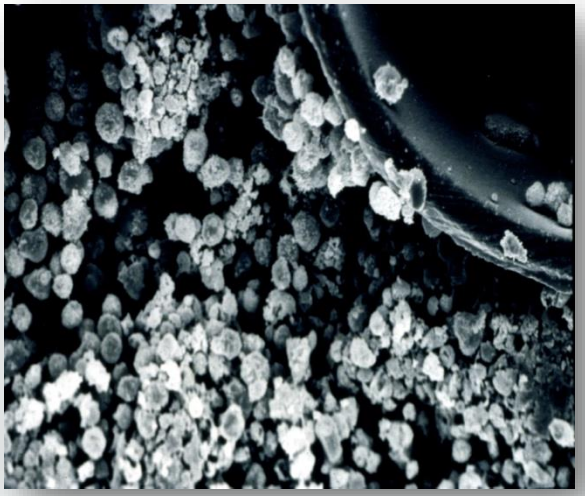


Hollow Fiber: How it Works



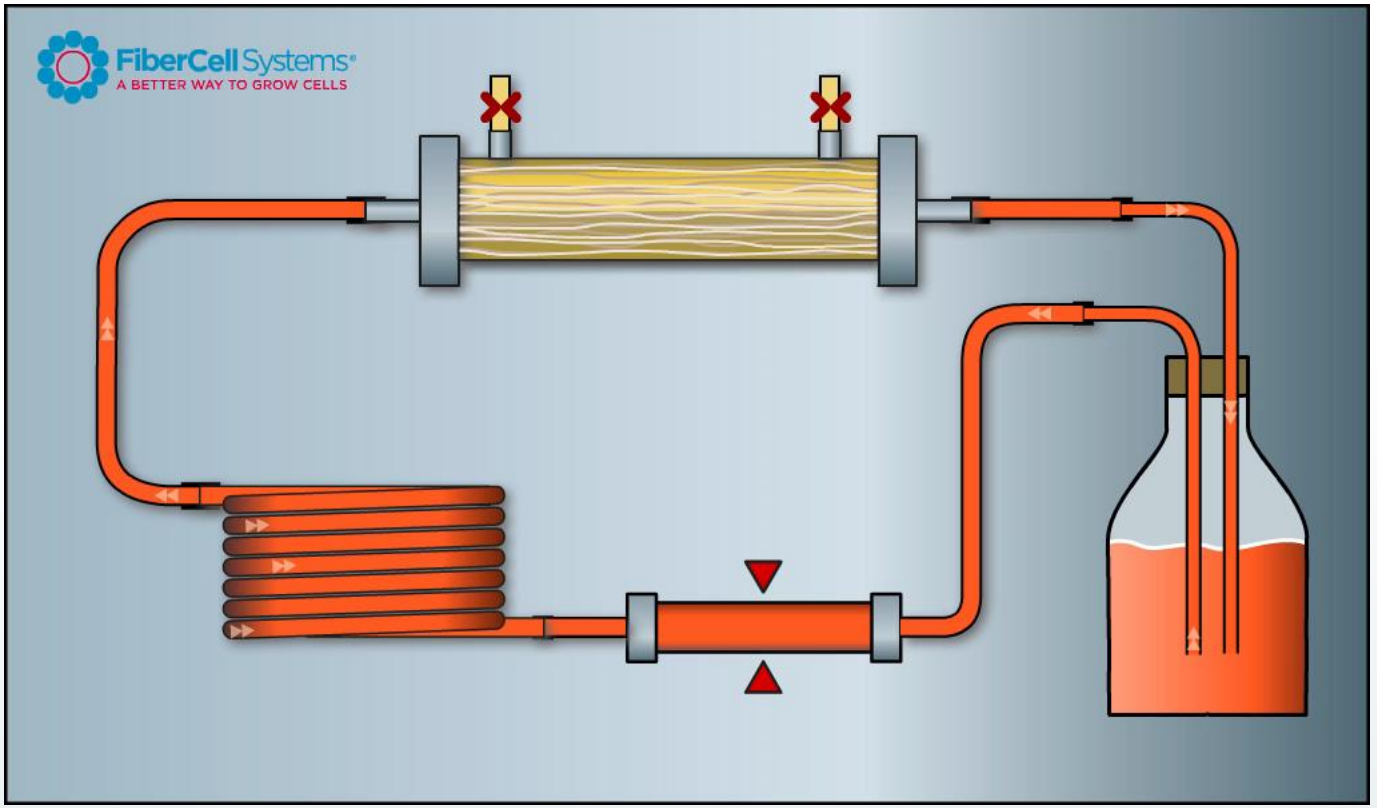
HF Culture of Lymphocytes

- 10^8 + cells/mL
- “Wavy” fibers optimized for suspension cells
- High cell density permits adaptation to lower serum concentrations and protein-free medium such as CDM-HD

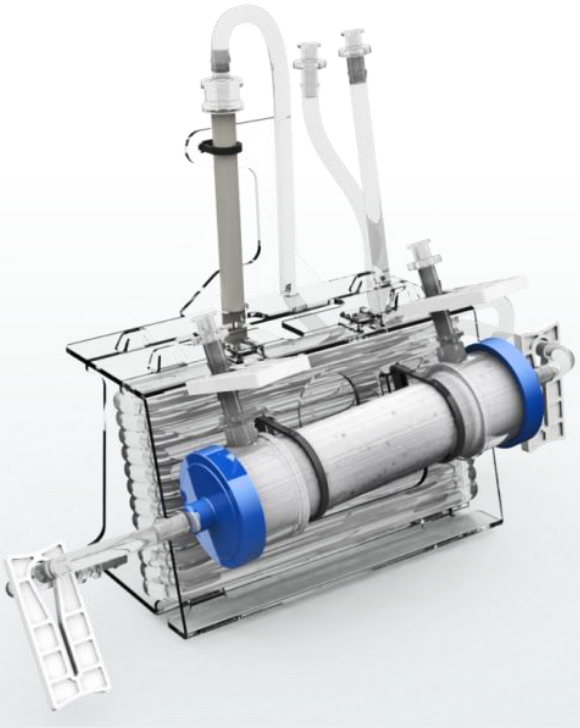


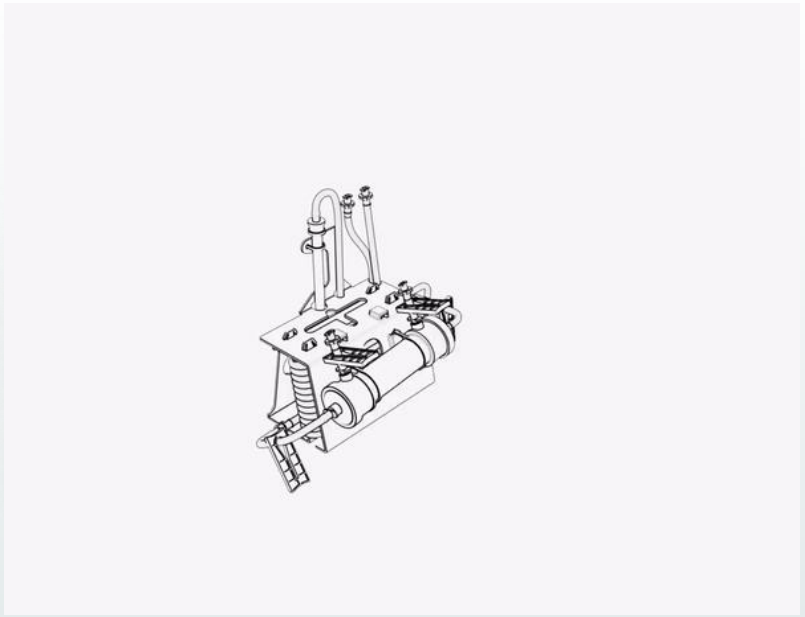
HFBR are Fundamentally Different in 3 Ways

1. Extremely high surface area/volume permits high density cell culture.
2. Cells are bound to a porous support, not a non-porous 2-D flask.
3. The molecular weight cut off (MWCO) of the fibers retains and concentrates secreted products.



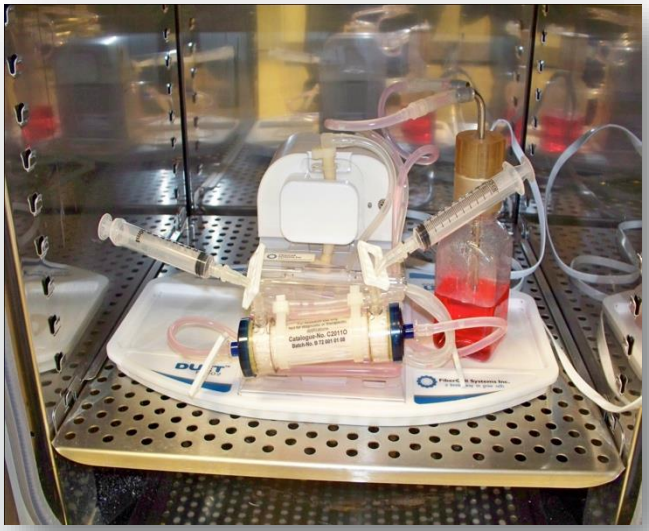
- Positive pressure displacement pump
- Silicone tubing for gas exchange
- Closed, bio-safe system





In the Laboratory

- Fits in any standard sized incubator
- Gas controlled by incubator
- Temperature controlled by incubator
- Thin cord for power



Working with the Cartridge

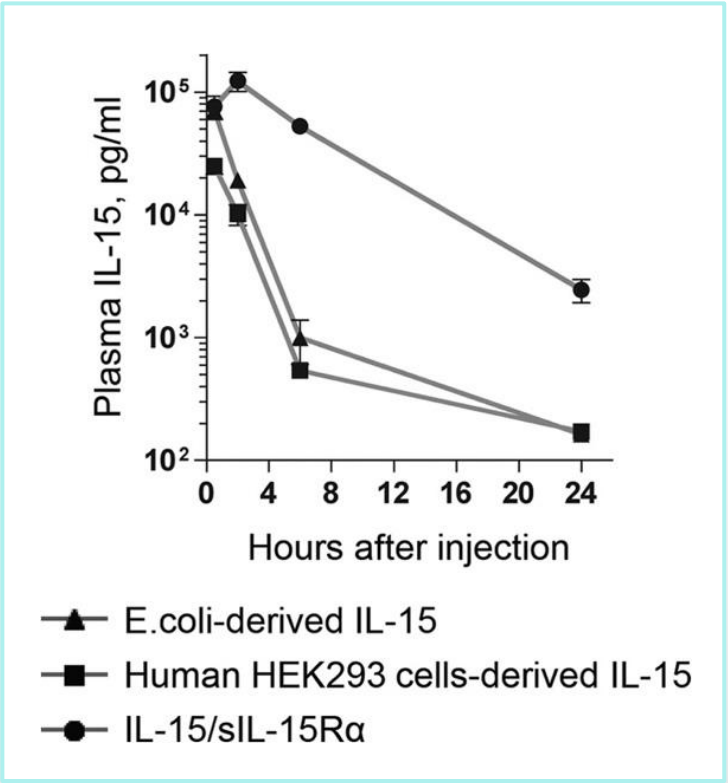
- Moves easily into hood
- Good sterile technique always a plus
- Maintenance only 15 minutes per day
- Harvest product and measure glucose consumption

HF Applications

- Monoclonal antibody production
- Recombinant protein production
- Conditioned medium
- Exosome production
- Endothelial cell culture under shear stress
- Cell co-cultivation
- *in vitro* toxicology

Mammalian Expression

- Improved solubility
- Correct bioactivity and immunoreactivity
- Longer serum half-life



"Difficult-to-Express Proteins

- Highly Glycosylated
- High level of post-translational modifications
- Low levels of secretion

  – IL15 receptor complex
  – BITE: Bi-specific T-Cell Enhancers
  – TRIKE: Tri-Specific Natural Killer Enhancers

  Structures not found in nature

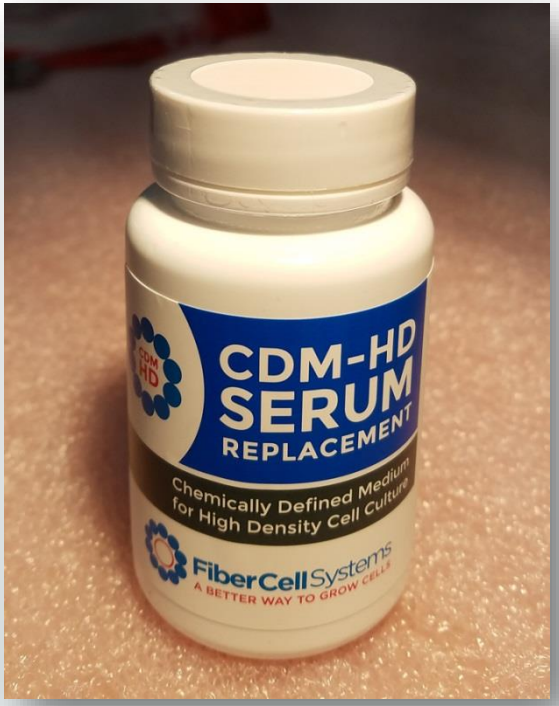

For Research use only!
Not for diagnostic or
therapeutic applications!
Catalogue-No. C2011
Batch-No. B 72 016 06 05

Advantages of Hollow Fiber Cell Culture

- Concentrated product
- Uniform and complete post-translational modifications
- Low apoptosis, less contamination with intracellular proteins and DNA
- Consistency of production over many months
- Protein-free medium (CDM-HD)

CDM-HD Serum Replacement

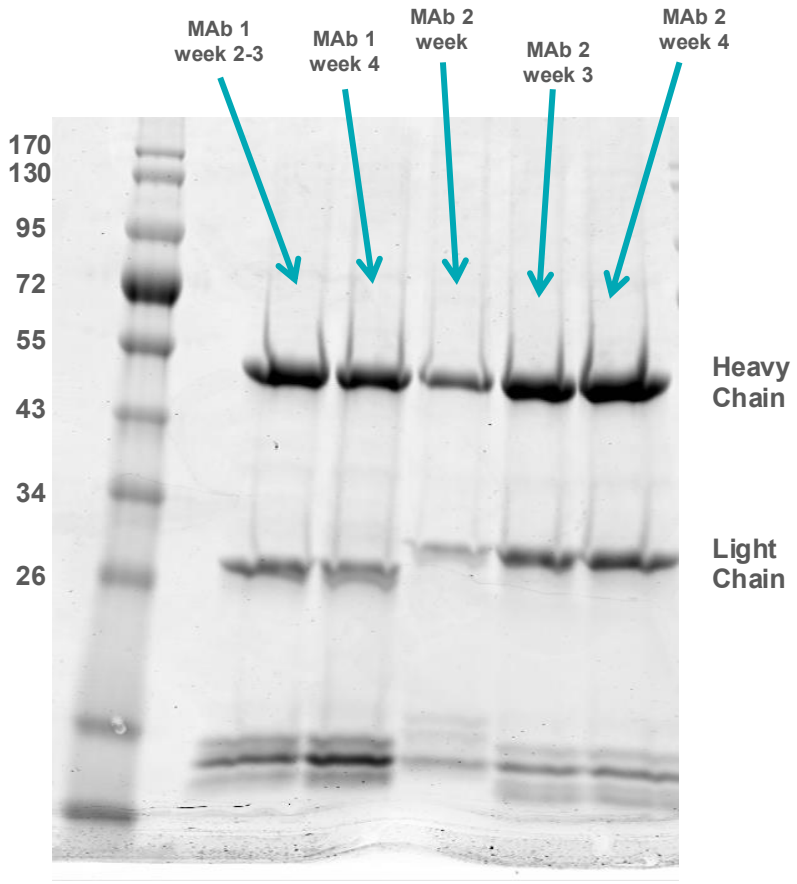
- Optimized and simplified for HFBR
- Contains no surfactants
- Chemically defined, protein-free
- cGMP compliant
- Lot-to-lot consistency
- Ship at ambient, store at 4˚C



Mab Production using CDM-HD

Mab 1:
- 168 mg in 60 mL volume, 2.8 mg/mL.
- 9 L of medium consumed, 3 weeks culture.

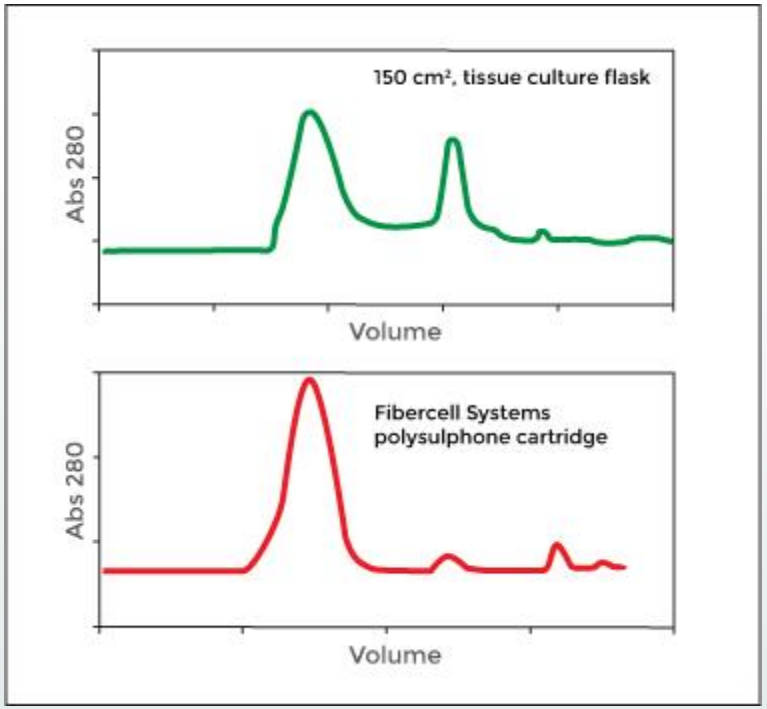
Mab 2:
- 159 mg in 70 mL volume, 2.3 mg/mL.
- 11 L of medium consumed, 3weeks culture.



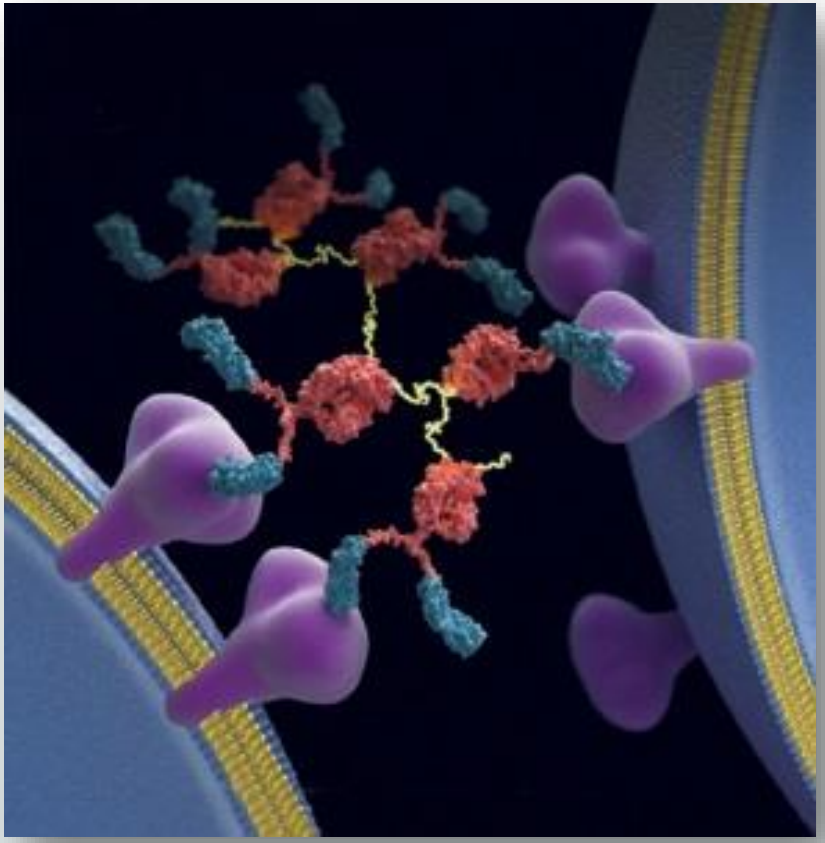
- TGF Beta diffuses out
- Mab trapped in ECS
- Easily adapt to SFM/CDM-HD
- Lower endotoxin
- .5 to 5 mg/mL conc.
- 5-100 mg per harvest
- Continuous production for over 6 months

Recombinant Protein Production

- Both suspension and adherent cell types
- 100x+ higher concentration
- Easily adapt to SFM
- Can provide improved protein folding



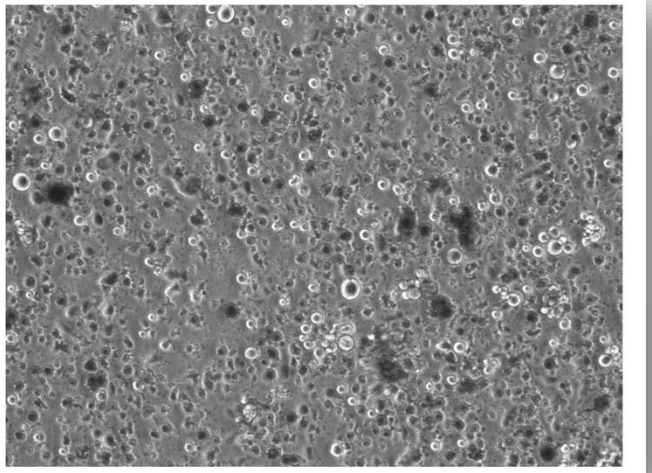
Journal of Biological Chemistry, Sept 2007

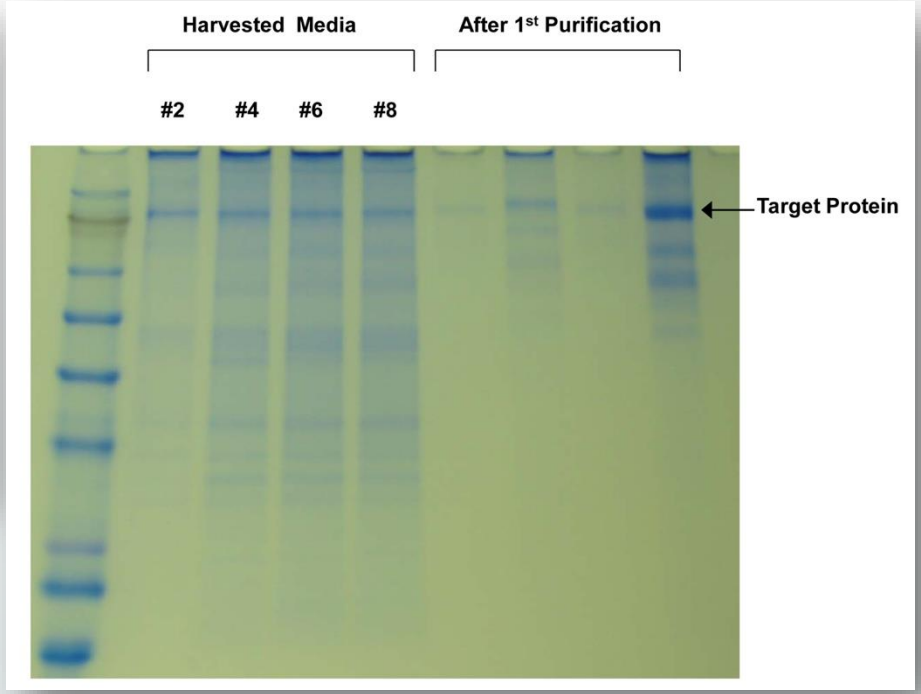


FiberCellSystems
A BETTER WAY TO GROW CELLS

www.fibercellsystems.com

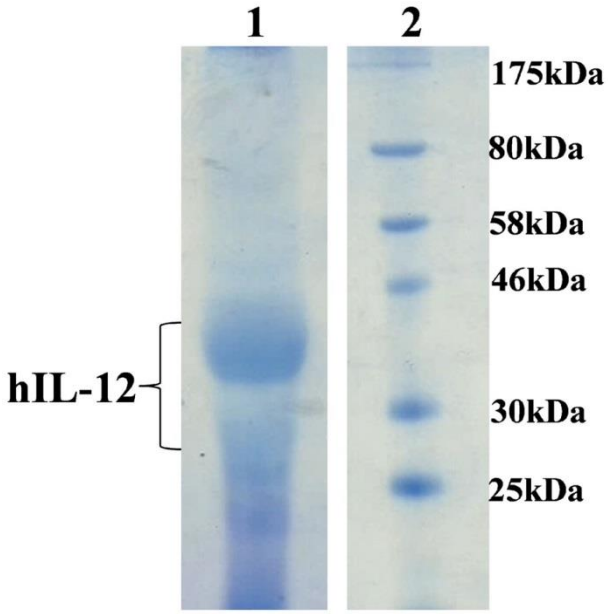
Raw Harvests from DG44 CHO Cell Line





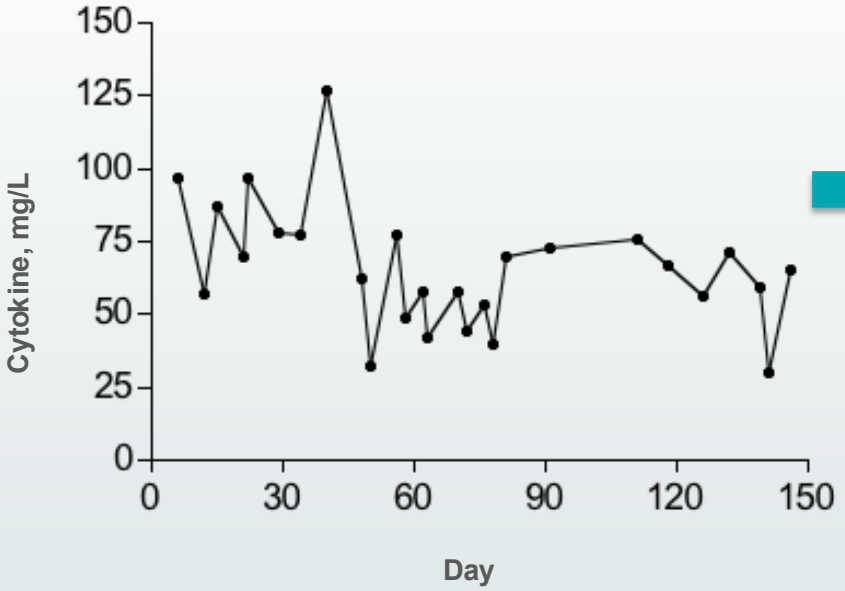
Recombinant IL12 Expressed in 293T Cells

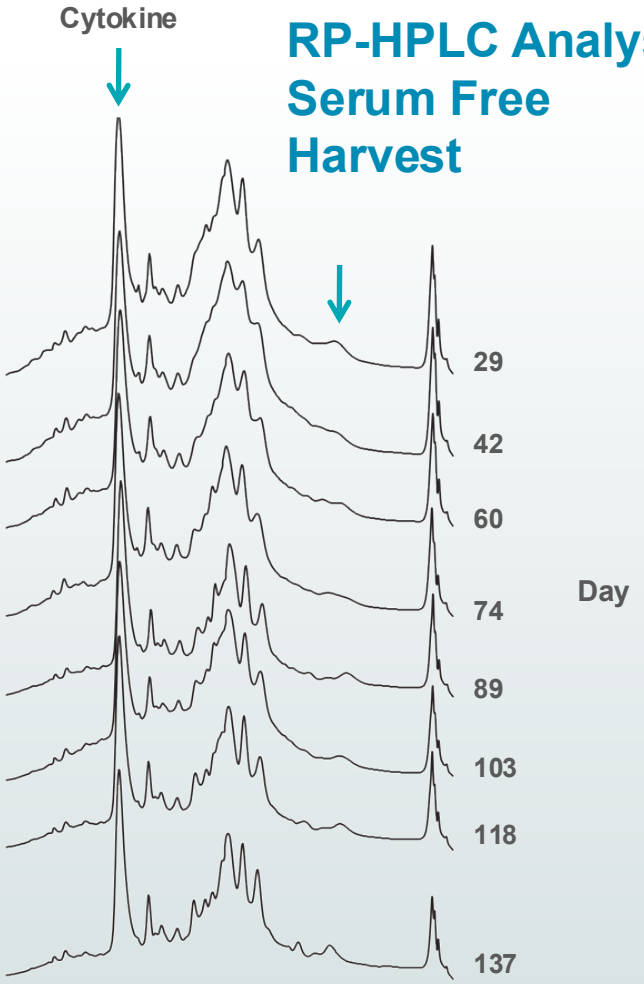
- Stable Native Conformation
- 25% of total protein in cell culture supernatant
- Critical inter-subunit disulfide bond is intact
- Produced using CDM-HD protein free medium



Stable IL15 RC Production in HFBR Over 5 Months

HFBR Production - mg/L

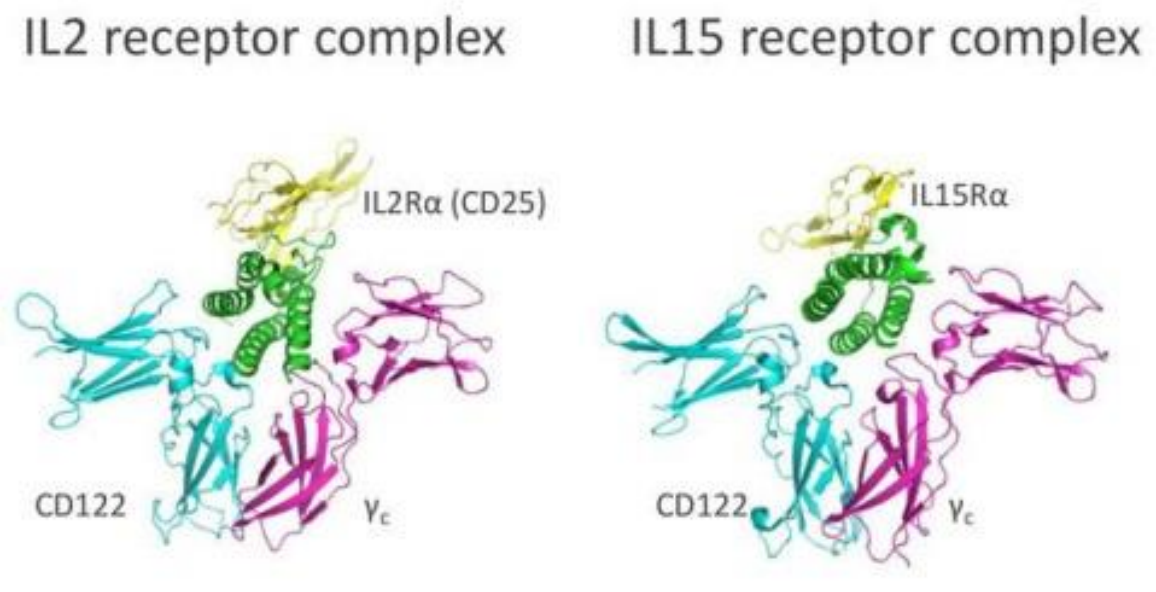




FiberCellSystems
A BETTER WAY TO GROW CELLS

www.fibercellsystems.com

IL15 RC is a Difficult to Express Protein



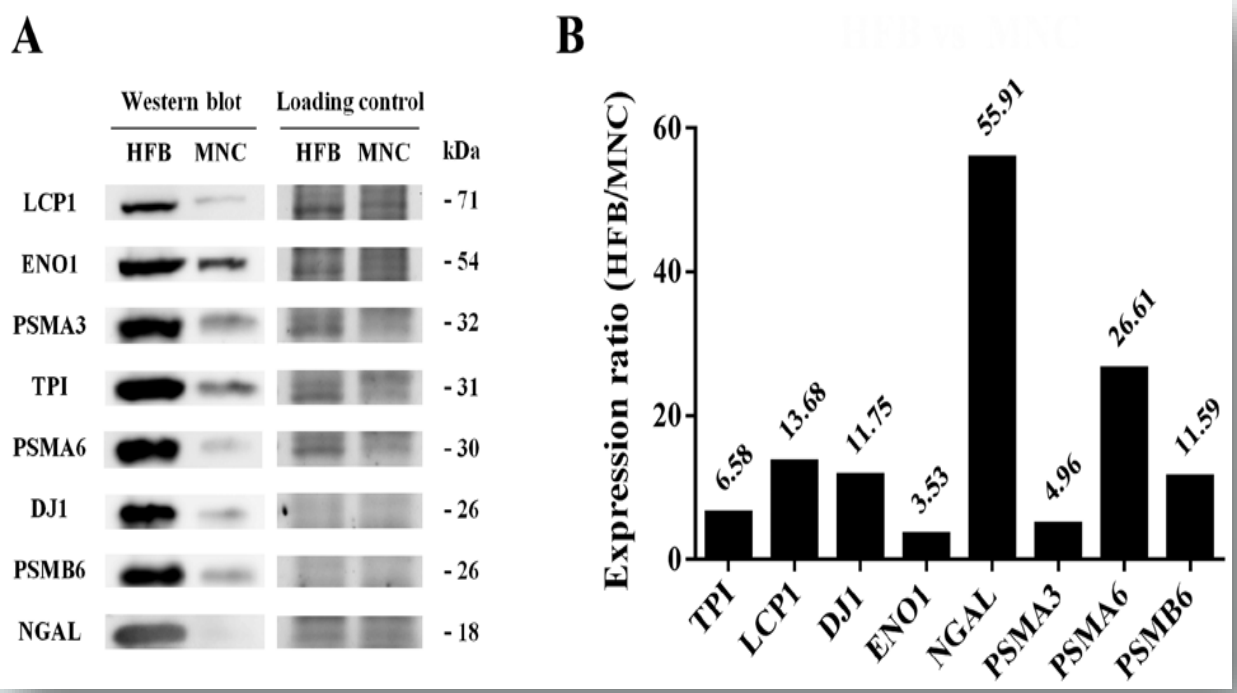
HFBR Equivalents

- A single harvest from C2011 can equal 20 roller bottles per day
- A single harvest from C2018 can equal 200 roller bottles per day
- 10 mL harvest volume can equal 1 liter of cell culture supernatent

Plastic Waste Generated by $1x10^9$ Cells

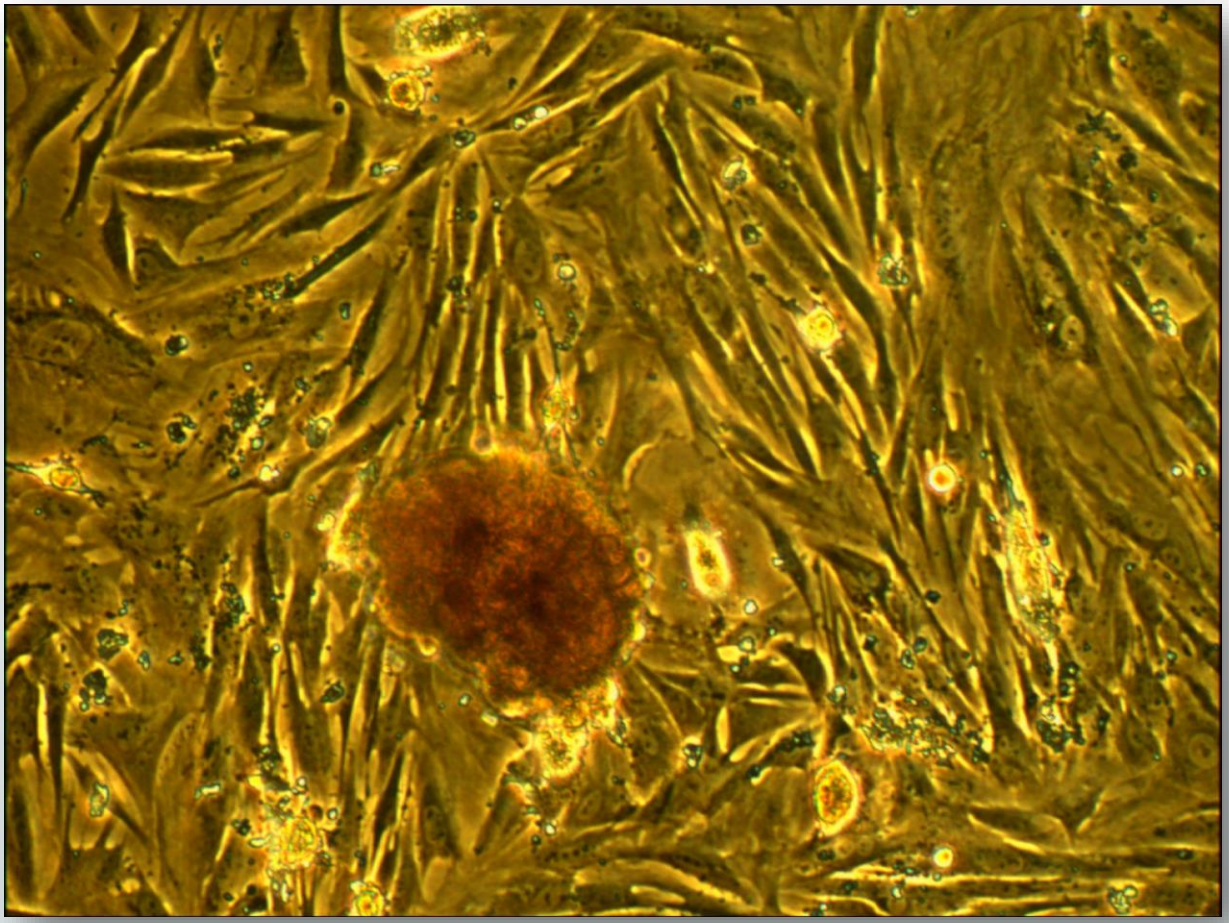
Secretome Analysis for Cancer Biomarker Discovery

Comparison of Flask vs. HFBR



Pulsatile Perfusion of Placenta

Placental Co-Culture



FiberCellSystems
A BETTER WAY TO GROW CELLS

www.fibercellsystems.com

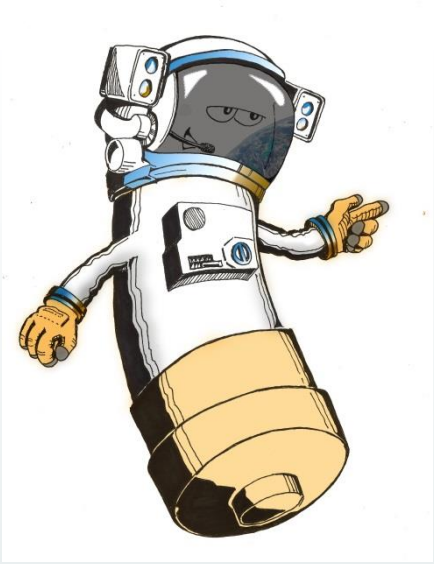
Harvest vs. Flask

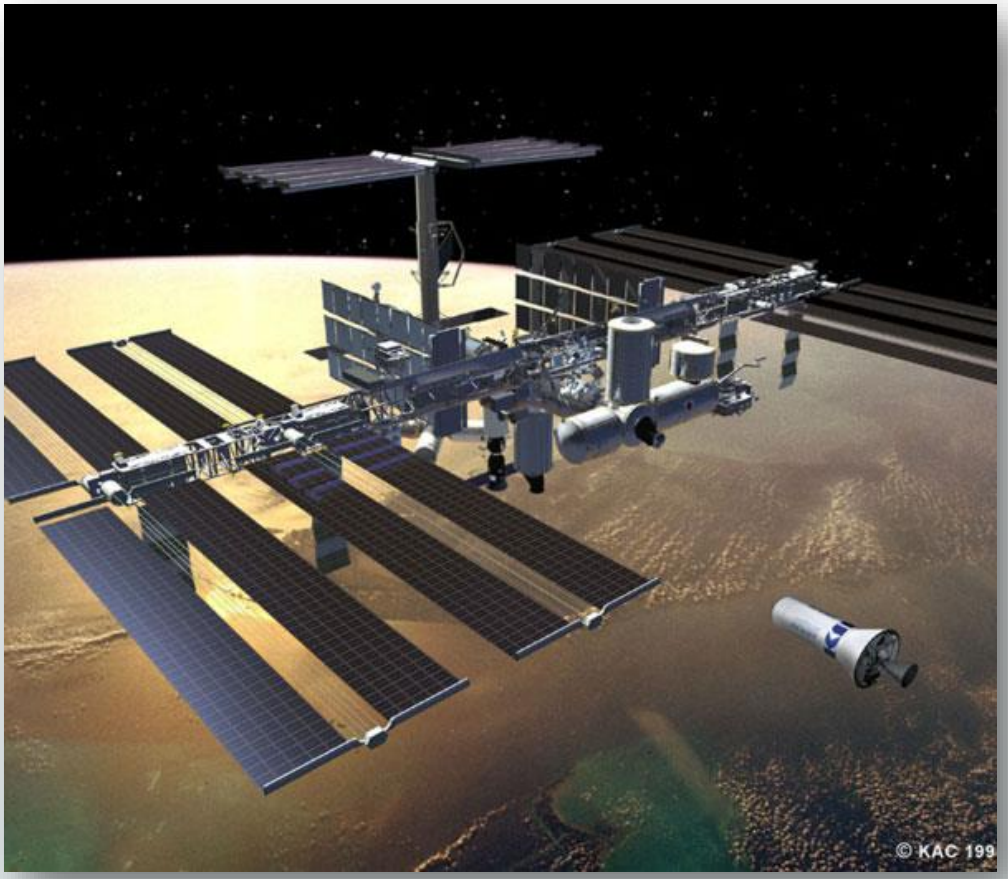
Phenotype	ECS Harvest	Flask
CD 45	4%	1%
CD 34	0%	0%
CD 133/2	2%	0%
CD 31	3%	48%
CD 13	6%	83%
CD 105	43%	99%
CD 73	18%	99%
CD 90	5%	96%
CD 14	23%	4%
NANOG	0%	0%

Summary

- Hollow fiber bioreactors are the method of choice for the culture of 10^9 to 10^{11} cells
- Ideal for producing 100 mg to several grams of Mab, 10 to 100s mg of recombinant proteins
- Concentration of products 10 to 100x higher than with conventional methods
- The most *in vivo* method for culturing cells over long periods of time
- Saves time, space, purification costs

FiberCell Systems HFBR in Space

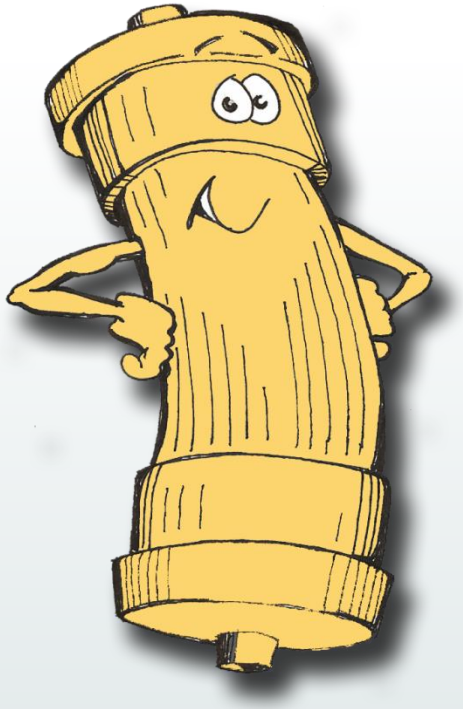




FiberCellSystems
A BETTER WAY TO GROW CELLS

www.fibercellsystems.com

Thank you.



FiberCellSystems
A BETTER WAY TO GROW CELLS

www.fibercellsystems.com