FiberCell Systems Inc.
a better way to grow cells

Production of Large Quantities of Mouse and Rabbit Monoclonal Antibodies using the FiberCell Systems® Inc. Bioreactor

By John J. S. Cadwell

The FiberCell® Systems hollow fiber bioreactor allows the production of about 50 – 200 mg of antibody per month from standard hybridomas using the small/medium sized reactor. The advantage of this system is that antibody production can be performed in chemically defined media (no proteins at all) resulting in pure hydridoma generated antibody with no antibody contamination from fetal bovine serum or mouse ascities. The product also is harvested in a concentrated form 500 - 3000 µg/ml and production of the antibody can be accomplished in a standard CO^2 incubator.

Equipment

FiberCell® Systems Duet	(cat # P3202)
FiberCell® Systems Reservoir Cap (sterilized)	(cat# A1006)
- *fits standard plastic media bottle*	
FiberCell® Systems CDM-HD Serum Replacement	(cat#CDM-HD-1)
FiberCell® Systems Medium Cartridge	(cat# 4300-C2011)
Hylcone Antibiotic/antimycotic soln	(cat# SV30079.01)
20ml syringes	
Alcohol wipes	
70% ethanol spray bottle	
Centrifuge tubes	
Sterile pipettes	

Setup

At all times you must practice aseptic technique and liberally use alcohol and wipes to ensure sterility.

Day 1. Attach the reservoir cap to a 500 ml bottle of sterile PBS. Fill the ECS with PBS. Attach to cartridge (see instructions enclosed with bioreactor) and insert into pump (Duet Pump).

Day 2. Change out bottle and replace with a new 500 ml bottle of DMEM + 1% antibiotics. Drain bioreactor ECS and refill with new media (pull through with vacuum pressure using syringe). Replace the syringe.

Day 3. Change out bottle and replace with new 500 ml bottle of DMEM +1% antibiotics + 10% CDM-HD. Drain bioreactor and refill with new media using a new sterile syringe (pull through with vacuum pressure using syringe).

Day 4. Change out bottle and replace with new 500 ml bottle of DMEM +1% antibiotics + 10% CDM-HD. Drain bioreactor and seed with cells. Harvest cells from 4 x75cm^2 flasks (minimum 10^8), concentrate cells into a pellet and re-suspend in 20 ml of the conditioned medium + 1 ml of FBS (helps to establish cells in reactor).

Day 5-7. Check glucose level (change bottle when glucose is below 2.0 g/l). Change bottle to a 1000 ml DMEM +1% antibiotics + 10% CDM-HD. Ensure that glucose rate is at least 1 gram of glucose consumed per day.

Day 8 - 30. Daily monitoring of glucose levels (when near 2.0 g/l) change bottle and harvest cells. Ultimately you want to set up a Monday/Wednesday/ Friday schedule for harvesting and bottle changes.

Mondays – Bottle change and supernatant harvest. Exchange DMEM bottle with a new 1L bottle (with antibiotics and CDM-HD). Gently tip the apparatus to one side. Draw all the media from cartridge into a 20 ml syringe and put the harvested media into a centrifuge tube. Replace the syringe with a new sterile syringe. Draw fresh media into the bioreactor using vacuum pressure. Aspirate back and forth.

Wednesday - Bottle change and supernatant harvest. Exchange DMEM bottle with a new 1L bottle (with antibiotics and CDM-HD). Gently tip the apparatus to one side. Draw all the media from cartridge into a 20 ml syringe and put the harvested media into a centrifuge tube. Replace the syringe with a new sterile syringe. Draw in fresh media using vacuum pressure. Aspirate back and forth.

Friday - Bottle change, cell and supernatant harvest. Exchange DMEM bottle with a new 1L bottle (with antibiotics and CDM-HD). Gently tip the apparatus to one side. Draw all the media from cartridge into a 20 ml syringe and put the harvested media into a centrifuge tube. Replace the syringe with a new sterile syringe. Draw in fresh media using vacuum pressure. Aspirate back and forth. Gently tip the apparatus to one side and draw all the media containing a huge number of cells from cartridge into a 20 ml syringe. Replace the syringe with a new sterile syringe. Draw in fresh media using vacuum pressure. Aspirate back and forth. Gently tip the apparatus to one side and draw all the media containing cells from cartridge into a 20 ml syringe. The number of time you want to do this depends on the growth characteristics of your cells, usually twice is sufficient to reduce cell numbers to allow the cartridge to go unattended over the weekend, while still getting a good yield of antibody on Monday.

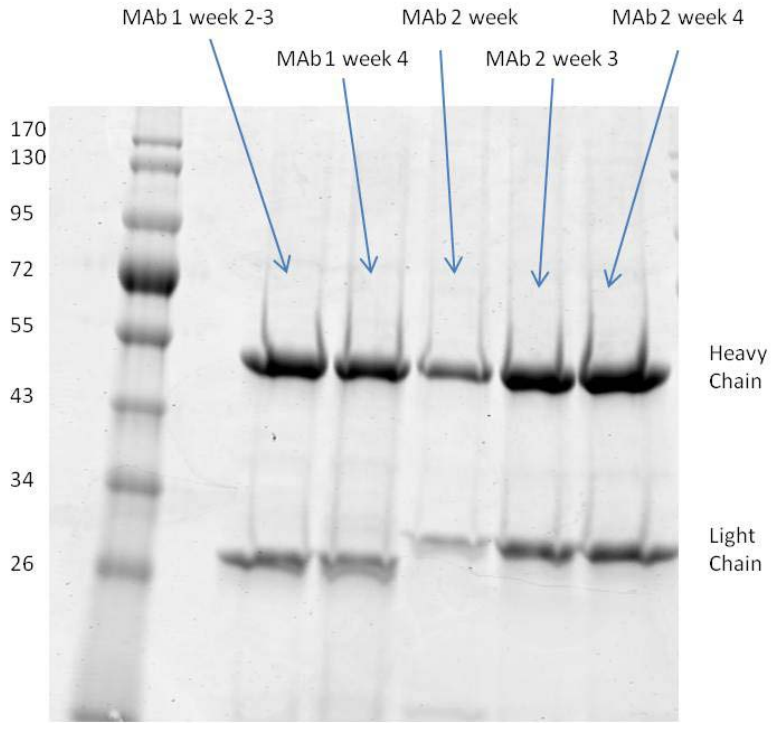
Supernatants – Spin at 1000 g to pellet cells, harvest supernatant, discard cell pellet. Pool supernatants from Monday, Wednesday and Friday harvests. Place into dialysis tubing and dialyze against 2 L PBS with 0.3 M EDTA, repeat, then dialyze against PBS (or whatever buffer you wish). Yields are usually 12 ml per harvest (36 ml week) at an antibody concentration of 1 - 3 mg/ml (total yield 36 – 108 mg/week) once the system is stabilized (usually day 14).

Cells - The supernatant and cells collected from the two washes on Friday does not contain significant antibody. However, you can place the cells pellet into sterile PBS and allow it to secrete overnight and you will get another 1 – 3 mg of antibody from those cells.

Case Study 1: Mouse Monoclonal Antibody Production

As an example, two mabs produced in DMEM + CDM-HD media were treated as above. The dialyzed supernatants had their protein concentration quantified and purity check on a 12% SDS - PAGE gel.

Mab 1 – 2.6 mg/ml week two and three harvest (40 ml)
Mab 1 – 3.2 mg/ml week four harvest (20 ml)
Mab 2 – 0.8 mg/ml week two harvest (25 ml)
Mab 2 – 3.2 mg/ml week three harvest (20 ml)
Mab 2 – 3.0 mg/ml week four harvest (25 ml)



Data courtesy of Dr. Erin Bromage, U. Mass. Dartmouth

Case Study 2: Rabbit Monoclonal Antibody Production

Rabbit monoclonal antibodies can be especially difficult to produce. Secretion levels can be so low as to be nearly undetectable in flask culture, typically 1 µg/ml or lower. Culture conditions have not yet been optimized for this relatively new cell type. In the example cited below nearly 20 mg of antibody were produced over a 2 week period of time. RPMI with 10% CDM-HD and 2% FBS was found to provide the best performance for this particular clone. RPMI is generally not considered to be a good medium to use with hollow fiber bioreactors systems due to it's low glucose concentration (2.5 g/L) CDM-HD adds an additional 1 g/L of glucose. Medium changes were performed when glucose levels were below 1.5 g/L. The cartridge was run for several months under these conditions.

Day	[ng/ml]	[mg]
1	140581	2.11
2	213741	3.21
4	98588	1.48
7	149001	2.24
10	135789	2.04
14	265100	3.98
18	60841	0.91

Production of a rabbit monoclonal antibody utilizing the FiberCell C2011 cartridge. 16 mg of antibody in a volume of 140 ml of harvest. 6 L of medium were consumed over a two week period of time.

Data courtesy of Tong Ming Fu and Daniel Freed, Merck and Co.