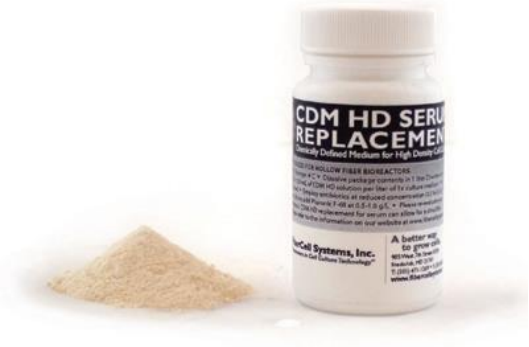
FiberCell Systems Inc.
a better way to grow cells

CDM-HD Usage Instructions

CDM-HD (chemically defined medium for high density cell culture) is a chemically defined, protein free, animal component free cell culture supplement that is designed to replace fetal bovine serum in standard basal cell culture mediums such as DMEM. CDM-HD is designed specifically for high-density cell culture and should be used in the FiberCell® Systems hollow fiber system for best results. Cells cultured in a hollow fiber bioreactor are typically 100X the density of cells grown in flasks or other conventional cell culture devices. Cells grown at these densities are able to auto-support their growth with secreted conditioning factors and can be grown efficiently with a simplified serum replacement like CDM-HD. It is important to know both what is IN CDM-HD and what is NOT.

CDM-HD contains:



1) A complex formulation of vitamins, micronutrients, amino acids and other basic chemicals
2) A significant amount of free iron
3) The equivalent of 10mM HEPES per liter when added at 10% to medium
4) The equivalent of 1 gram of glucose per liter when added at 10% to medium

CDM-HD does NOT contain:

1) Protein of any sort. Therefore there are neither attachment factors nor iron transport proteins.
2) Surfactants like pluronic F-68
3) Animal or plant hydrolysates or anything else of an undefined nature.

CDM-HD may also be used in spinner or shaker flask culture, if desired. There are no surfactants added to CDM-HD because in hollow fiber there is little or no shear on the cells. Spinner and shaker flask do contribute to shear on the cells and it is recommended that a surfactant such as pluronic F-68 be added to the medium to protect the cell membranes. Because the cells are at a lower density you may need to use a higher concentration of CDM-HD such as 15-20%. Since there are no attachment factors you will need to seed some adherent cells in the presence of serum, allow them to attach and then switch to CDM-HD.

For use in the FiberCell® Systems hollow fiber bioreactor system it is recommended that the cells be inoculated into the cartridge in the presence of at least 5% serum in the cell innoculum. Once the cells have reached high density in the cartridge, as defined by a glucose uptake rate of 1 gram per day, simply switch out the medium in the reservoir and ECS for medium containing 10% CDM-HD. You may observe a slight lag in growth of the cells for a day or two but no sequential adaptation is required.

Protein purification using CDM-HD

Since CDM-HD is protein free you may be able to simplify your purification protocols. This should be based upon your own direct experience along with an analysis of the harvests from your particular cell line. When purifying monoclonal antibodies from cell culture supernatants using Protein A, you should be aware that there is a higher than normal amount of free iron present to compensate for the lack of transferrin (a protein) in the medium. This amount of iron can sometimes cause precipitation if the harvested supernatant is placed directly into a phosphate buffer system such as might be used for Protein affinity purification. There are some alternative binding buffers that do not contain phosphate that can be used with Protein A. FiberCell® Systems has been provided some specific information by Bio-Rad Laboratories on an appropriate binding buffer and Protein A kit that is compatible with CDM-HD. Products from other companies may also be used.

One option would be to add Bio-Rad's MAPS binding buffer to enhance binding of the antibody to the Protein A gel. This buffer is optimized for mouse iG2A, but works well with most other antibodies (except rat).

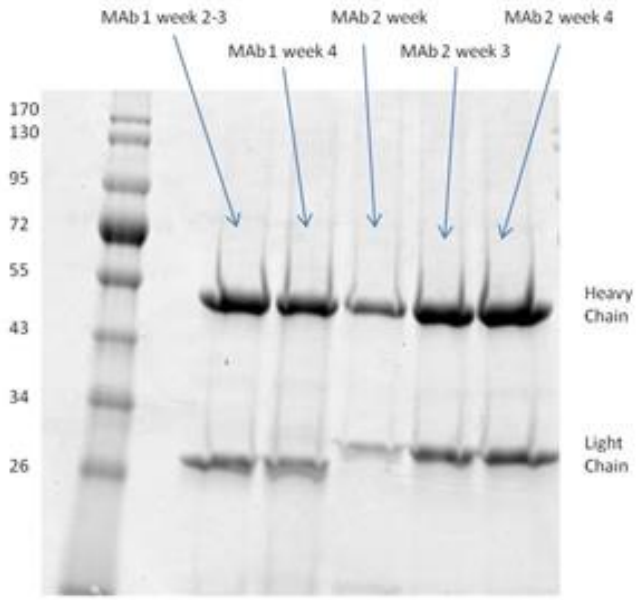
Buffer formulations:
Binding Buffer: 3.2 M NaCl in 1.6M glycine, pH9,
Elution Buffers: 100 mM Na citrate, pH3,
Regeneration Buffer: 50% methanol/water,

The MAPS binding buffer is used by either adding the dry salts to a dilute sample, or it can be dissolved in water and then add two parts buffer to one part sample:

Catalog numbers for the binding buffer:
153-6161, buffer salts to make 5 liters, $784
153-6159 Affi-Gel Protein A MAPS II kit, which includes 5 ml Protein A, an empty column, and buffers to purify 500 mg of antibody. $442,

Bio-Rad also offers two other Protein A supports, which are pressure stable and can be run on a chromatography system.

Feel free to contact Bio-Rad technical support at 1-800-424-6723, option 2, or lsg_techserv_us@bio-rad.com.



Above: Unpurified supernatant from harvest using CDM-HD. Data courtesy of: Dr. Erin Bromage c/o the US Veterinary Immune Reagents Network

Preparation of CDM-HD for use.

1. Fill a mixing vessel with distilled or WFI quality water, to 90% of the desired total volume of media. For example, for one liter of CDM-HD, fill the vessel to 900mL.
2. Add CDM-HD to the water while gently stirring. Rinse the inside of the package with some of the remaining 10% water to remove any remaining powder.
3. Finish adding remaining water to bring to a volume of 1 liter and mix until completely dissolved. This should take 15-30 minutes at room temperature.
4. CDM-HD should be pH adjusted to 6.8 if necessary, using 1NaOH or 1N HCL.
5. Filter into a sterile container by membrane filtration, using a 0.2 micron filter.
6. Store at 4-6 C.
7. CDM-HD is provided at 33.36 grams to make one liter.

Notes:

1) Do not heat CDM-HD to speed dissolution.
2) pH should be adjusted to no higher than 6.8 for best storage. CDM-HD is stable as a liquid at 4 degrees for at least 6 weeks.
3) Do not freeze CDM-HD once it has been reconstituted.
4) CDM-HD powder should be stored at 4 degrees C and has a shelf life of at least one year.
5) Add CDM-HD to classical media such as DMEM High glucose at a final concentration of 10%.