Hybridoma culture

Supplies
Hybridoma cells
BDCell mAb medium quantum yield (cat. no. 220511)
Fetal bovine serum
Nutridoma CS (Roche)
Integra bioreactor (called Cellline 1000 Flask, from Wheaton)

Method
Grow the hybridoma cells in T25 flask in BDCell Mab medium + 10% FBS + nutridoma (1:50 from stock). Nutridoma makes the cells grow much faster, but isn't essential at this stage. Keep cell densities between 0.5 million/mL and 3 million/mL. Expand to T75 flask as the cells grow. Once you have around 2 - 3 T75 flasks you're ready to inoculate the bioreactor. It takes about a week or so to reach this point from a frozen aliquot of ~5 million cells. Freeze backup aliquots of cells if needed, so you can recover if there's contamination.

Inoculate the chamber as in the manufacturer's instructions. You don't need as many cells as they recommend. Seed with around 35 mL at 2 million per mL or higher. Chamber medium composition is BDCell mab medium + Pen/Strep, no other additives. The cell medium (lower chamber) should be the chamber medium supplemented with 1:50 nutridoma and 15% FBS. Adding FBS to the chamber medium does not improve yield or growth rate.

Track cell viability. At first, cells will grow slowly as they adapt to the chamber. Then they'll saturate and start to die. Once cell viability reaches 50% (by trypan blue staining) you're ready to harvest. Don't rely on the medium color to tell you when its time – it will turn orange while the cells are still viable and actively secreting antibody.

Once cells reach 50% viability, harvest all the cells and then put 5 mL back in, together with 30 mL fresh cell medium. The volume of harvest is typically about 40 mL, since the cell chamber swells slightly during culture. Change the chamber medium at the same time as the cell medium. Spin the harvested cells and freeze the supernatant for later.

After 3-4 days you're ready for another harvest. This approach is much faster than the 2 week harvest, and the yields are about the same. Recent yield averaged 40 – 50 mg antibody per harvest, which is almost 100 mg per week.

FLAG resin preparation

Supplies
FLAG peptide (DYKDDDD)
FLAG-Cys peptide (DYKDDDDGGC)
Iodoacetamide resin (Pierce)
CNBr sepharose 4B (GE)

Procedure
Make a peptide column using FLAG-Cys peptide and iodoacetamide resin. Use a saturating amount of peptide and just follow the instructions. This is easy, and you only have to do it once. You can store the resin + azide at 4 degrees for months or years. Experimentally measured capacity is 11 mg antibody per mL of resin. 20 mL of resin is plenty.

Pool harvested supernatant from 3 - 4 or harvests, and then flow load over the peptide column (add 2 mM calcium before loading). Wash with HEPES-buffered saline + 2 mM CaCl2. After washing, elute with 100 mM citrate pH 3. Use citrate, not glycine. If you use glycine you have to dialyze for days afterward to remove it, since it contains a primary amine that prevents coupling to the resin. If you use citrate, it's ready right away. After elution add HEPES (not tris, no primary amines) to bring the pH up to 7.5 or so. You will need a lot of HEPES to do this. The elution is generally kind of slow, so be patient. You'll get about 150 - 200 mg of protein from four good harvests, which will make about 15 - 20 mL of FLAG resin.

To make the resin, just follow the manufacturer's protocols. Use 12 mg antibody per 1 mL swelled resin. You can measure uncoupled antibody by A280. At this coupling density, you should achieve complete coupling of antibody to resin overnight. I quench with 20 mM tris pH 8 afterward to ensure no activated groups remain.

Store FLAG resin in PBS or HBS with 2 mM sodium azide. With proper handling it will work for at least ten uses, potentially much more. The most important thing is to wash with acid after each use, and then neutralize promptly. I also filter all dirty samples prior to loading, which helps immensely in avoiding long-term slow clogging with repeated use.