Care and Feeding of mAb104 cells

To thaw cells:

1. Thaw bullet quickly (in hand or water bath) and wipe down outside with Ethanol.
2. Add contents of bullet to 10 mls media+10% FCS (see below) and spin 5 min at 1 K.
3. Aspirate off supernatant and resuspend cell pellet in 5-10 mls media+10% FCS.
4. Transfer to a well of a 6 well plate or a T25 flask and let recover for several days in incubator

To maintain/grow cells:

Once growing well (should approximately double every 24 hrs.), split every 2-3 days and keep at density of 0.3-1.2 million cells/ml. These can be expanded as needed.

To make antibody stock:

After cells have recovered from thaw and been expanded up to approximately 50 mls, start to

ween off serum. I do this by first splitting 1:2 with media+5% serum, then 1:2 with media + 2% serum, or no serum. The goal is eventually to get at least 200 mls of cells in a final serum concentration of 2-5%. The cells will slow down with decreased serum, so it's best to get them close to final concentration before you drop to less than 2%. The decrease in serum just reduces competing protein concentration in antibody stock.

Once cells are at desired volume and serum concentration let grow until as dense as possible

(~2 million cells/ml). Then put cells and media in 50 ml. conical tubes and spin 5 min at

1 K. Pool supernatant and sterile filter through 0.2 micron filter (this will reduce the chance of crap growing in your antibody stock). Also can add 0.1% sodium azide. (Discard cells that will be sick at this point).

To Freeze cells:

1. Grow approximately 50-100 mls cells in media with 5-10% FCS to a density of ~1 million/ml.
2. Spin down cells in sterile conical tubes. 5 min at 1 K. Aspirate supernatant.
3. Resuspend cells in 1 ml Freezing media (see below) for every 10 mls of original culture.
4. Aliquot 1.5-2 mls in cryovials. Put in –20 degrees for 1-2 hours then move to –80 freezer.

Media

RPMI 1640 w/ glutamine (we get our from Fisher/Mediatech cat# MT 10-040-CV) supplement with Penicillin, Streptomycin (100 U each final) and Glutamine (2 mM final) and desired concentration of FCS (fetal calf serum)

Freezing Media

50% "media" (see above)
40% FCS (regardless of how much serum is in your "media")
10% DMSO