## Reagents

1. 1M Tris-HCL pH8.0
2. 2. Sodium azide 10% (1000x)
3. 3. 0.22uM bottle top filters
4. 4. Centrifuge tubes (rated up to 5000xg)
5. 5. Protein A/G or antigen column
6. 6. Media: DMEM 4.5g/l glucose, 10% FBS, pen/strep
7. 7. Flasks
8. 8. pH paper

## Protocol

1. Break out hybridoma cell line:
   * Typically cells are rapidly thawed at 37oC
   * Place cells in 10mls of DMEM, 10% FBS, pen/strep
   * Spin at 1500xg, 5min, room temperature
   * Aspirate supernatant
   * Resuspend in 5mls of media, plate in t25
2. Hybridoma's are typically are loosely adherent
   * When they are at low density, the grow better in conditioned media
   * as such, until cells are ~50% dense, iIaspirate media to remove dead cell debris and replace media (5mls)
   * live cells have a bright halo, dead cells look like screwed up paper
   * if the cells are not adherant, I remove them from the flask, spin them down at 1000xg, 5min, and replace the media. I replate the cells in the conditioned T25.
3. As cells become more dense I typically splti them 1:3 every 2-3 days
   * unless the % of dead cells increases, I don't spin them down and change the media
   * everytime you change the media you throw out antibody
   * The cells are loosely adherent, thus you should try an pipette them off the substrate
4. For the best antibody production, it’s best to grow the cells to high density and then split them
   * From a T25 to a T75
     + Grow to high density in 10mls
     + Dilute this to 50mls in a T75
   * From a T75 to a T175
     + Grow to high density in 50mls
     + Dilute to 200mls in a T175
   * To split to 5x T175s
     + Dilute 50mls (of 200) to 250mls in a 4xnew T175
     + Place new media (250mls) in the flask you recovered the cells from (there will be enough adherent cells to seed this flask)
5. Once you have the desired volume of cell culture supernatant that you need - do NOTHING - this is the trick
   * typically we get back 4-16mg of purfied antibody per liter of cell culture supernatant, as such your needs  will dictate "the desired volume"
   * for ~7-10 days check the cells daily
   * they will continue to increase in density, the media will acidify, and eventually the cells will start to die
   * do NOT feed the cells in this phase
   * when ~80% of the cells are dead, progress to step 6
6. Harvest cell culture supernatant into centrifuge tubes
   * Neutralize with 1M Tris-HCL pH8, to between pH7.5 and 8
   * Check the pH with pH paper
   * Add sodium azide to 0.01%
   * Remove cell debris by centrifugation, 5000xg 15min, 4oC
   * Decant supernatant
   * filter though 0.45uM filter into a sterile container
   * store at 4oC until required
   * Typically, we store cell culture supernatant for under 1 week before purification