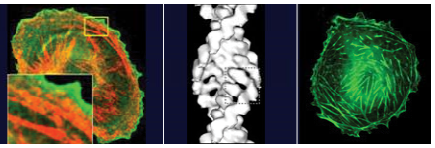

Hybridoma cell culture and antibody purification

1. Thawing Hybridoma cells
    a. Cells are more delicate than normal tissue culture cells
    b. Thaw cells rapidly, then transfer them to 5 ml warm RPMI 1640 medium containing 10% FBS and 1:100 P/S (Mediatech MT30002CI)
    c. Spin down at 800 x g for 5 min
    d. Remove supe and resuspend gently in 5 ml warm RPMI 1640
    e. Plate onto 6cm tissue culture plate
    f. If low on vials of cells, freeze some down in freezing medium

2. Splitting cells
    a. Cells need to be split 1:5 every 3 days or so.
        i. This is usually done when medium turns from pink to orange
            1. Density should be around 1 x 10^6 cells/ml when splitting
            2. Do not let cells get overgrown
        ii. Cells are not adherent, should look round, smooth and quite large
    b. Split cells into larger plates every-time by simply adding cells to fresh medium
        i. 5 ml into 20 on large 15 cm plates
        ii. 25 ml into 100 ml in 75 cm^2 flasks
        iii. 125 ml into 5x 100 ml in 5x 75 ml cm^2 flasks
    c. Never throw away any supernatant as it contains the secreted antibody

3. Antibody Purification
    a. Collect all cells, ~500 ml, and spin down at 2.5k, 5 min 4 °C
        i. Collect supernatant and filter with 0.22 µm filter top
    b. Equilibrate 1 ml HiTrap Protein G HP column (GE healthcare 17-0404-03) in cold room
        i. Cold, filtered 10 ml PBS
    c. Apply supernatant to column at 1.5 ml/min
    d. Wash column with 20 ml PBS
    e. Elute antibodies with 5 ml 100 mM Glycine pH 2.5
        i. Collect 0.5 ml fractions into tubes containing 25 µL 1M TRIS pH 9.5
        ii. Gently mix tubes immediately after elution is complete to neutralize pH
    f. Wash column with 20 ml PBS
        i. Store in 20% EtOH at 4 °C
    g. Run fractions on SDS PAGE gel
        i. Should get two bands, 30 & 50 kDa
        ii. Some samples may be yellow due to pH, they will turn blue when loaded onto gel

    iii. Can also check by abs@ 280nm, but since only 10 samples, you will run them all on a gel anyway
h. Collect fractions containing high concentrations of antibody
    i. Dialyze in 2 L PBS over night
    ii. If time permits, change buffer once to remove excess glycerol
i. Dialyze in 500 ml of PBS/50% Glycerol for at least 5 hours
j. Measure antibody concentration
    i. Should get ~300 µLs or 5.0 mg/ml
k. Store at -20 °C