Adsorption of polyclonal antibodies with antigen-negative cells

This is a simple method to "clean up" polyclonal antibodies that detect non-specific bands in cell lysates by immunoprecipitation or immunoblotting. The idea is to incubate the antiserum with lysates of cells that lack the antigen; this will allow adsorption of antibodies that recognize material present in the lysates but leave behind antibodies to the antigen of interest. In this case, the "lysate" is in the form of an intact or fixed cell. Thus the non-specific antibodies are removed by simple centrifugation. What is left in the supernatant is an "adsorbed antiserum". It's a very old trick used in the old days to generate antibodies specific to a given cell type or to a polymorphic antigen, like MHC molecules.

1. Grow desired cells that lack the antigen of interest. The more cells you have, the better.

   Optimal: 10^8 lymphocytes or 2-5x10^7 adherent cells per ml of antiserum.

2. If the cells are adherent, remove them from the dish/ flask by treatment with PBS/ 0.5mM EDTA (note – **DO NOT USE TRYPSIN**).
   - wash cells with PBS
   - incubate with 2 ml/ 10 cm dish or T75 flask of Ca^{2+}/Mg^{2+}-free PBS containing 0.5 mM EDTA
   - when cells start to lift off the dish, add 8 ml of complete medium (with serum) and pipette up and down
   - pellet cells by centrifugation at ~500g for 5 min

3. Wash cells 1X with PBS

4. Fix cells by resuspending them in a small volume of PBS/ 2% (w/v) formaldehyde or paraformaldehyde. Fix on ice for 15-30 min.

5. Wash cells 2X with ice cold PBS

6. Permeabilize cells by resuspending them in a small volume of ice cold PBS/ 0.2% saponin/ 0.1% BSA. Incubate for 15 min on ice.

7. Wash cells 3X with ice cold PBS. Pellet cells and remove all supernatant.

8. Add serum to permeabilized cell pellet and resuspend the pellet.

   - incubate on a rotator at 4˚C for 60-120 min.

9. Pellet cells and collect the supernatant as your adsorbed antiserum.

10. If you have enough cells, repeat steps 8 and 9 with a second batch of cells to doubly deplete junk.

11. Aliquot and label your adsorbed antiserum appropriately. Store at 4˚C or -80˚C for long-term.