SOP: AB102

QC of Antibodies

Materials and Reagents:
1. Serological pipettes
2. Western blot assay supplies
3. Cryovials
4. Cryovial storage container

Protocol:
1. _____ Obtain a small aliquot of culture supernatant or IgG purified antibody (see SOP:AB100 or AB101).

2. _____ Run SDS-PAGE with 1ug of target protein (note 1).

3. _____ Perform western blot assay and vary the dilution of the antibody. For culture supernatant: make 1:20, 1:50, 1:100 dilutions. For IgG purified: make 1:500, 1:1000, 1:2500 dilutions (note 2).

4. _____ Once the titer is determined, run another SDS-PAGE with 1μg of the protein of interest and 5ug each of CFP, cytosol, cell wall and cell membrane.

5. _____ Run a western blot the determined titer of antibody (note 3).

6. _____ If performing QC on IgG purified antibody, run a gel of 1 μg of antibody and silver stain (note 4).

7. _____ Aliquot antibody in cryovials and store at -80˚C (note 5).

8. _____ Scan western blot and Silver stain (if applicable).

9. _____ Fill out QC sheet titled: TB Contract Antibody QC Sheet.

10. _____ See SOP:RM001 for Adding Material to Inventory for further direction.

Notes:
1. Run several lanes of the same protein, this will be used to determine the titer of the antibody. See SOP: SP007 on running polyacrylamide gels.
2. See SOP: SP011 for running a western blot. Cut the nitrocellulose into individual strips for each dilution. If the titer does not fall into these ranges adjust accordingly and repeat. If testing a monoclonal be sure to use anti-mouse IgG secondary, if testing a polyclonal be sure to use anti-rabbit IgG secondary.
3. For polyclonal antibodies, if titer is determined using a recombinant protein back the titer off by one dilution for the QC on subcellular fractions. For example: Titer for anti-ESAT6 tested against recombinant ESAT6 was determined to be 1:10000, the QC for this antibody would be done at 1:5000.
4. See SOP:SP012 for performing a Silver stain.
5. For culture supernatant: if titer is >1:50 make 0.5 ml aliquots (default), and if <1:50 make 1.0 ml aliquots (default). For IgG purified: make 0.5 mg (default) and 1.0 mg aliquots. IgG purified antibody should be freeze dried by lyophilization see SOP: SP004.