

SOP: AB102.1
Modified: 7-24-13

QC of Antibodies

Materials and Reagents:

1. Serological pipettes
2. Western blot assay supplies (SOP: SP011)
3. Cryovials
4. Cryovial box
5. Cryovial labels (Cryotags # MINI-1700)

Protocol:

- 1._____ Obtain a small aliquot of rabbit sera, culture supernatant, or purified antibody (SOP: AB100 or AB101).
- 2._____ Run SDS-PAGE with 1 µg of target protein (SP007) in several lanes with a marker for each sample. Perform a western blot transfer (SOP: SP011) and cut the nitrocellulose into individual strips.
- 3._____ Develop the individual western blot strips using varying dilutions of the antibody, being sure to use the appropriate secondary antibody:

For culture supernatant: start with 1:20, 1:50, 1:100 dilutions.

For purified antibody: start with 1:500, 1:1000, 1:2500 dilutions.

For rabbit polyclonal sera: start with 1:1000, 1:5000, 1:10,000
- 4._____ Repeat steps 1-2 as necessary adjusting the titer if it does not fall within the above suggested range.
- 5._____ Once the titer is determined, run another SDS-PAGE with 1µg of the protein of interest and/or 5 µg of the appropriate subcellular fractions for the antibody being tested.
- 6._____ Run a western blot using the determined titer of antibody. 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.
- 7._____ If performing QC on purified antibody, run a gel of 1 µg of antibody and silver stain (SOP: SP012).
- 8._____ Aliquot antibody in cryovials and store at -80°C. For culture supernatant: if titer is >1:50 make 0.5 ml aliquots, and if <1:50 make 1.0 ml aliquots. For purified antibody: make 0.5 mg (default) and 1.0 mg aliquots. Purified antibody should be freeze dried by lyophilization see SOP: SP004. For rabbit polyclonal sera: made 0.1 ml or 0.25 ml aliquots as appropriate.
- 9._____ Scan western blot and Silver stain (if applicable).
- 10._____ Fill out QC sheet titled: TB Contract Antibody QC Sheet.
- 11._____ See SOP: RM001 for Adding Material to Inventory for further direction.