

SOP: AB101

IgG Purification of Monoclonal Antibodies

Materials and Reagents:

1. Serological pipettes
2. Disposable poly-prep Chromatography Columns
3. Protein G-sepharose in 20% ethanol suspension (P-3296 Sigma)
4. Phosphate Buffered Saline (PBS) pH 7.4 or 10mM Ammonium Bicarbonate (Ambic)
5. Pasteur pipettes
6. Clean rollerbottle
7. 2L culture supernatant of antibody to purify
8. 4°C Cold Room
9. 0.2M Glycine
10. Slide-A-Lyzer Dialysis Cassette 3500 MWCO (Pierce 66110)
11. Lyophilizer
12. Thin rubber tubing
13. 5 ml Falcon collection tubes
14. Western blot supplies
15. Silver Staining supplies
16. SDS-PAGE supplies

Protocol:

1. ____ Obtain 2L culture supernatant of antibody that is being purified (note 1).
2. ____ Set up column by filling the column with PBS.
3. ____ Add 0.6-0.8ml of protein G-sepharose slurry (note 2).
4. ____ Using a Pasteur pipette flush the slurry until the bubbles disappear.
5. ____ Continue to add PBS so the column doesn't dry out.
6. ____ In a 4°C cold room, place the culture supernatant above the column (note 3).
7. ____ Place an empty clean 2L roller bottle below the column.
8. ____ Fill the column with culture supernatant.
9. ____ Connect the rubber tubing from the culture supernatant roller bottle to the top of the column.
10. ____ Connect another section of rubber tubing from the bottom of the column to the empty roller bottle.
11. ____ The proper flow rate should be ~ 1 drop every 4-6 seconds (note 4).
12. ____ Check on the flow rate every couple of hours.
13. ____ Repeat steps 7-12 two more times (note 5).
14. ____ Wash the column with 50 ml of PBS (note 6).
15. ____ Set up 6 falcon collection tubes, and add 0.75 ml of sterile 0.1M PBS to each tube (note 7).
16. ____ Elute with 0.5 ml of 0.2µm filtered, 0.2M Glycine HCL, pH 3.0 per collection tube.
17. ____ Run 1-2 ul of all the fractions on a SDS-PAGE, and Silver Stain (note 8).

18. _____ Pool the fractions that contain purified antibody and dialyze against PBS or Ambic, depending how the antibodies will be stored, using the Slide-A-Lyzer Cassette for 24 hours (note 9).
19. _____ Run a BCA on the dialyzed sample to determine antibody concentration (note 10).
20. _____ Run a SDS-PAGE gel of the purified samples and a western blot of the target antigen. Develop the western blot with dilutions of 1:500, 1:1000, and 1:2500 of the purified antibody (note 11).
21. _____ Aliquot the antibodies in 0.5mg and 1.0mg aliquots as determined by a BCA assay (note 12). Leave a 100ul sample of the purified antibody to perform QC.
22. _____ Freeze dry by lyophilization if needed (note 13).
23. _____ See SOP: AB102 for QC of antibodies to continue.

Notes:

1. See SOP: AB100 for cell line maintenance.
2. Protein G- sepharose is for IgG1 antibodies. Protein A sepharose can be used if the antibodies isotype is not IgG1 but most monoclonal antibodies are IgG1.
3. The culture supernatant must be above the column for gravity to drive the supernatant through the column.
4. It might be necessary to suck on the tubing connected to the culture supernatant to start the flow.
5. Be sure to not let the column run dry. When the top 2L culture supernatant bottle is empty, just switch bottles, so that the full one (with the flow though) is now above the column and the bottle that was originally full is now below the column.
6. This step can be done at room temperature. Discard the wash.
7. 10mM Ambic may also be used to neutralize the purified antibodies that are coming off the column. The buffer depends on how the antibodies will be stored. Use PBS if the antibodies will be freeze dried by lyophilization and use Ambic if they will be stored in solution.
8. See SOP: SP007 running polyacrylamide gels and SP012 performing a Silver Stain.
9. If there is more than 12 ml of solution use 3500 MWCO dialysis membrane.
10. See SOP: SP003 on BCA protein assay.
11. See SOP: SP011 running a western blot. Load 1-2 ug of pure protein or 5-10ug of subcellular fractions per well. It is best to make multiple wells containing the protein, so this can be used to titer out the antibody. If the titer doesn't fall into this range repeat the western blot until the proper titer is determined. For purified IgG antibodies the titer shouldn't be lower than 1:500. If it is, the purification should be repeated with new culture supernatant or a western can run be on the flow through to see if the antibodies did not bind to the column.
12. Default quantity for purified antibodies in 0.5mg.
13. See SOP: SP004 for use of lyophilizer.