Protocol 102_Antibody purification using "Gammabind Plus Sepharose" column (big column) and the BioRad fraction collector

Purification

1. If not in use the column and the system are "stored" in 20% EtOH.
2. Equilibrate column with cold PBS (stored in the cold room) for 60-90min at a flow rate of 3ml/min (180-270ml).
3. Calculate how long it will take to load the hybridoma supernatant you want to purify the antibody from (Volume of supernatant/flow rate). Standard flow rate is 3ml/min. Do NOT use a higher flow rate ever! If you want to run it over night make sure the flow rate is low enough to ensure that the column does not run dry!
4. Wash column for 60-90min with cold PBS.
5. Bound antibody is eluted with 0.1M Glycin (pH 2.7) at a flow rate of 3ml/min. Press F1 (Engage) on the fraction collector. The collector head will now move to the start position "1" on the rack. Switch to "Program" mode and press "List of methods". Open "Test1". Press "Run". With this method the fractionb collector will collect 40 fractions (each 3ml) at a flow rate of 3ml/min (~ 2h). After fraction 40 is collected, Glycin will continue running elution is now collected into the waste bottle (in order to remove residual antibody/protein bound to the column). Run Glycin until 200-300ml is collected into the waste bottle.
6. Equilibrate the column with cold PBS for 60min at 3ml/min.
7. If the column is being use again right away, proceed with the next batch of hybridoma supernatant (see step 3).
8. If the column is not being used again right away, run 20% EtOH for 60min at a flow rate of 3ml/min.
9. Turn off the pump and the UV

Dialysis of eluted antibody fractions

1. Using method "Test1" the most concentrated fractions are usually found between fractions 20-35.

2. Measure A280 to determine which fractions contain high concentrations of antibody.
3. Pool all fractions that contain high concentrations of antibody for dialysis.
4. Dialyse the pooled fractions using either:
   Slide-A-Lyzer Dialysis Cassette (3-12ml) or SnakeSkin Pleated Dialysis Tubing (3.7ml/cm) depending on the volume of antibody.
5. Fill the dialysis Cassette/Tube with the pooled antibody fractions.
6. Prepare two big 2L Erlenmeyer flasks filled with 2L of PBS (does not have to be sterile PBS).
7. Put Dialysis Cassette/Tube into the Erlenmeyer flask, add a magnetic stirrer, and put the flask on the stirrer in the cold room. Keep second flask in the cold room as well.
8. After 12h change the dialysis Cassette/Tube into the second Erlenmeyer flask and prepare a third fresh flask with 2L PBS and put it into the cold room.
9. After another 12 hours change again and dialyse again for the last 12 hours.
10. Recover the dialysed antibody solution from the Dialysis Cassette/Tube and transfer into a 50ml Falcon tube.
11. Sterilize solution using a 50ml syringe with a 0.2µm filter attached to it. Filter into a new sterile 50ml Falcon tube. Do this in a laminar flow hood!
12. At this point either go on with step 13, or put properly labeled Flacon tube into the clean fridge.
13. Dilute antibody solution 10x and determine the antibody concentrations as follows:

$$[Ab] = \frac{OD_{A\,280} \bullet 10}{1.4}\, mg/ml$$

11. Make 3ml aliquots of the antibody in 5ml Polypropylene Round-Bottom Tubes (352005) and store in -80°C freezer.