# Affinity Column Preparation

1. Prepare 1 ml of packed beads by washing them 4 times in 10 ml of d-$H_2O$ in a 15 ml tube. The beads are Act. Ultrogel 22 AcA from IBF, and stored in the coldroom. The protein binding site is via glutaraldehyde groups. 2 mls of beads from the vial in the coldroom equals 1 ml of packed beads upon centrifugation.

2. Then wash the beads 4 times in 10 ml of 0.1M NaBorate (pH 8.5) + 0.01% SDS made as:
   0.1M NaOH (8g/2l)
   pH with solid boric acid to 8.5
   Add SDS to 0.01% (1 ml of 20 % SDS/2l)

3. Add eletroeluted antigen to the beads after the final wash and removal of supernatant. The volume of the antigen to react with the beads should be ~1-2 ml. Optimal amount of protein is 2-5 mg, though as little as 200 μg will work.

4. Rotate overnight at room temperature in the 15 ml tube on a nutator.

Next day,
5. Wash away any uncoupled protein by 4 washes of 10 ml in PBS (pH 7.4). Save the first supernatant in case the protein did not bind to the beads.

6. Occupy all unused glutaraldehyde sites with 2 ml of 0.1M lysine in PBS. Mix with beads and rotate in the 15 ml tube for at least 5 hours at room temperature.

7. Wash away lysine by 4 washes of 10 ml each of PBS.

8. Add PBS to the beads and pour them into a small plastic column. Run PBS through the column to pack the beads, and store the column in the presence of PBS + 10mM $NaN_3$ at $4^oC$.

**To purify antibody**

9. When ready to add antibody, wash the column with ~20 ml of PBS. Lower the level of PBS to right above the beads. Never let the beads dry.

10. Add ~5 ml of antibody serum to the column and let it continuously flow through the column for at least 2 hours. Use a pump to make a circular flow from the bottom to the top of the column.

11. Wash away any contaminating proteins with PBS until the $A_{280nm}$= or < than 0.005. This will take 10-30 ml of PBS through the column. To read the absorbance, blank against PBS alone and read 1 ml aliquots as it drips from column.

12. Now place the column containing the bound antibody into the coldroom. All subsequent steps are done at $4^oC$. Let equilibrate to temperature for at least 10 min.

13. Prepare elution buffer of:
    0.2M glycine-HCl and chill in coldroom prior to addition to column. This buffer is made by making 0.2M glycine and pH to 2.8 using concentrated HCl.

14. Place 0.3 ml of 1M $K_2HPO_4$ into 15 eppendorf tubes. This will be used to immediately neutralize the antibody as it is eluted from the column. The antibody will denature at pH 2.8 over time, so it is important to neutralize as soon as possible.

15. Lower PBS in cooled column to right above the beads.

16. Add 10 ml of glycine-HCl solution to the column.

17. Elute by gravity and collect 0.7 ml fractions into the eppendorf tubes. So total volume in each tube is 1 ml.

18. Read the $A_{280nm}$ of each fraction and pool the fractions containing the majority of the antibody protein. Usually the antibody will elute in fractions 2-4.

19. The pooled peaks can be stored this way at -20$^{o}$C, or you can directly proceed to the dialysis step.

20. Wash the column extensively with cold PBS and store the column in PBS + 10mM $NaN_3$ at 4$^{o}$C. The column can be used several times.

21. Place the pooled fractions in dialysis tubing, that was first washed in PBS for 1 hour prior to use. Dialyze against 500 ml of 50% glycerol in PBS in the coldroom overnight with stirring. This will concentrate the antibody.

Next day:
22. Remove the antibody from the bag and place in epp. tubes. Read the $A_{280nm}$ of the final antibody prep.

To determine the concentration of the antibody, use the following formula:

$$c=\frac{A_{280nm}}{\varepsilon l}$$

where $\varepsilon l$=1.45 ml/mg and c is the concentration

so the concentration is just the absorbance divided by 1.45, and this value is in mg/ml.