AFFINITY PURIFICATION OF ANTI-FCRN ANTIBODIES ON THE FCRN COLUMN

Christina Milburn 10/1/96

Rationale: The FcRn column is usually used for "functional" purification of IgG's, i.e. by binding at pH 6 and eluting at pH 8. The procedure described here was developed to separate anti-FcRn antibodies from contaminating IgG's present in the ascites. Therefore binding is done at pH 8.5 to prevent the FcRn on the column from binding to the Fc portion of the IgGs in the sample; only IgG's specific for FcRn should be able to bind, as in a typical affinity purification. They are then eluted at pH 2.5.

I used this method for the first time to purify the monoclonal antibody 1G3 further after running the ascites over the Protein G column (Notebook "FcRn mutants II", 1/10/96). Since then Jose has also used it, with the modification of adding NaCl to the buffers as suggested by Luis.

Column: Large FcRn column was made by Luis.
Capacity 10+ mg IgG.
Bed volume about 15 ml.
Flow rate 1 ml/min.

Buffers:

A. Binding: 50 mM sodium phosphate, pH 8.5
500 mM sodium chloride
0.05% sodium azide

B. Elution: 50 mM sodium phosphate, pH 2.5
500 mM sodium chloride
(no azide because it has a high absorbance at 280 at low pH)

Purification Run:

1. Equilibrate column with 50 ml binding (pH 8.5) buffer.
2. Make sure sample is at pH 8.5 and contains 500 mM NaCl; filter 0.2 μm.
Run sample over column.
3. Wash column with binding buffer until UV trace returns to baseline. Make sure to wash thoroughly since column tends to retain albumin.
4. Elute column with pH 2.5 buffer. Collect 5 ml fractions; add 250 μl/fraction 1 M dibasic sodium phosphate with 2% azide to neutralize the pH. This will give a final pH of around 6.5 (and final concentrations of 100 mM $NaPO_4$; 0.05%NaAzide). Fractions may be concentrated and the buffer changed as desired for storage.
5. Re-equilibrate the column with 50+ ml binding buffer, or if you will be storing the column after the run use: 50 mM $NaPO_4$, pH 6, with azide.