Purification of Antibodies

Protein A:

The buffers I use are the ones recommended by Pharmacia in their catalog. There are several Pharmacia HiTrap protein A columns in the lab; if one of these is used, the column can be run on the FPLC. If you are using a column that has been used for purifying an antibody other than the one you wish to purify, thoroughly wash the column with elution buffer to remove any old antibody that may have been left behind. I typically will run 100 ml elution buffer over a column and equilibrate it with 5-10 column volumes of wash buffer before I use it. The wash buffer is 20 mM $NaPO_4$, pH 7.0 with 0.05% azide; and the elution buffer is 0.1 M citric acid-NaOH, pH 3.0, also with 0.05% azide. Run your antibody sample over the column, wash with 5-10 column volumes wash buffer, and elute with 5-10 column volumes elution buffer. Collect the eluate into 1 ml fractions; the collection tubes should contain 100 µl 1 M Tris, pH 7.4 to neutralize the acid. Concentrate the fractions containing protein (dialyze into an appropriate buffer, e.g. PBS / 0.05% azide) and check the concentration by BCA assay and store at 4ºC. Wash the column with 5-10 column volumes of wash buffer and run more protein over it. Note: the elution peak from the column starts out sharply, but diminishes very slowly. Pharmacia claims the binding capacity of the HiTrap column is 20 mg IgG / ml of gel. I have never tested the column to see if this is true.

Protein G:

Exactly the same as protein A purification, except Pharmacia says to use 0.1 M glycine-HCl, pH 2.7 for the elution buffer.

FcRn column:

The FcRn column must be used in the cold room. The protein sample must first be brought to pH 6.0 with $NaPO_4$ (bring the concentration of $NaPO_4$ to 50 mM in the sample). Run the sample over the column; wash with 5-10 column volumes of 50 mM $NaPO_4$, pH 6.0; and elute with $NaPO_4$, pH 8.0. All the buffers should contain 0.05% azide. Collect the eluate into 1 ml fractions and concentrate those containing protein (dialyze into an appropriate buffer, e.g. PBS / 0.05% azide). Check the concentration by BCA assay and store at 4ºC.

Antibodies I Have Purified

34-1-2S:

This is a mouse IgG_{2a} that recognizes H-2 K^d and H-2 D^d MHC class I heavy chains. Purify on protein A columns. Warning: do not give this to Susan Ou to produce ascites without telling her that it will kill mice with the MHC haplotypes: $K^{d,b,s,r,q,p}$ and D^d.

M1/42.3.9.8.HLK (M1/42 for short):

This is a rat IgG_{2a} that reacts with all H-2 haplotypes if they are complexed with mouse β_2m. This MAb cannot be purified on protein A or G columns, and must be purified on the FcRn column.

H57-597 (H57 for short):

This is a hamster IgG that recognizes the β subunit of T cell receptors. Purify on a protein G column.

D4:

This antibody recognizes I-E^k complexed with a peptide. Purify on a protein A column.