PURIFICATION OF FCRN MUTANTS ON THE 1G3 COLUMN
(for mutants that won't bind to the rat IgG column)

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Buffers: 50 M NaPhosphate, pH 7.5/0.05 % NaAzide (binding)

50 mM NaPhosphate, pH 8.5/0.05 % NaAzide (wash)

50 mM NaPhosphate, pH 3.0 <u>without</u> NaAzide** (elution)

to neutralize fractions: 1 M dibasic NaPhosphate/2% azide

Flow rate: 1 ml/min (or less as convenient)

Column capacity: about 0.7 mg protein (bed volume about 5 ml)

Purification procedure:

1. a) Column should be in pH 7.5 buffer to start.

b) Make sure pH of harvest is 7.5 (or higher).

c) Run 0.5 - 1 L harvest over column.

2. Wash harvest off column with pH 7.5 buffer (10 - 20 ml).

3. Wash column with at least 60 ml pH 8.5 buffer (a bit over 10 column volumes).

4. a) Before eluting, add 125 ul of 1 M dibasic NaPhosphate/2% azide to each tube.

b) Elute column with pH 3 buffer; collect 5 ml fractions (15 fractions is fine).

c) This means the final buffer of the fractions will be 75 mM NaPhosphate with 0.05% azide. The final pH will be about 6.5. (At first I checked the pH of the pooled fractions before concentrating them with the pH meter, but the pH was consistently 6.4 - 6.6. Now I just check the pH of a few fractions with paper. If it's too acidic, shake the tubes to mix buffers.) The protein can be left in this buffer for storage.

5. Re-equilibrate the column with at least 60 ml of pH 7.5 buffer.

6. a) Check A280 of the fractions; peak starts in fraction 3-6 and comes off in 3-4 fractions (depending on how much liquid is left over the column bed).

b) Concentrate in a Centriprep30 and filter through a 0.2 micron spin-filter.

** It turns out that azide at pH 3 has a high absorbance at 280 nm (> 0.1), so it causes problems when using a UV monitor while eluting. If you're not going to do that, it's fine to have azide in the elution buffer (and not add any to the buffer for neutralizing the fractions). However because of this azide/low pH weirdness it seems to work best to make up a blank for the spec with the same buffer mix as is in the fractions.

Macintosh hard drive/Lab Information/Lab potocols/1G3 Column