PURIFICATION OF FCRN MUTANTS ON THE 1G3 COLUMN

(for mutants that won't bind to the rat IgG column)

As of 6/14/96

Buffer: 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 without NaAzide (elution)

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

1. Harvests:
   - Collect 2-3 times a week from cells grown in 10 cm plates
   - Filter through 0.45 micron filter to remove cell debris
   - Add EDTA to 1 mM final and NaAzide to 0.05% final, e.g. for 500 ml harvest:

     1 ml 0.5 M EDTA stock
     2.5 ml 10% azide stock

2. - Run 0.5 - 1 L harvest at a time, depending on yield (column capacity is about 0.7 mg protein).
   - Make sure pH of harvest is about 7.5.
   - Column should be in pH 7.5 buffer to start.

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

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

5. Elute column with pH 3 buffer.
   - Before eluting, add 125 ul of 1 M dibasic NaPhosphate with 2% azide to each tube to neutralize pH of the fractions as soon as possible.

   - Collect 5 ml fractions.

   This means the final buffer of the fractions will be 75 mM NaPhosphate with 0.05% azi
   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.)

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

6. Check the A280 of the fractions on the spectrophotometer.
   Concentrate those containing protein by spinning in a Centriprep - 30 to the smallest volume (about 0.7 ml). Leave them in the pH 6.5 phosphate buffer.
   Filter through a 0.2 micron spin-filter.

7. Check the A260, A280 and A320 of the concentrated protein.
   The 280/260 ratio is usually about 1.5.
   A280/1.4 is supposed to give the protein concentration for FcRn.
   Run a BCA asssay - this seems to give a concentration about 70-75% of that determined the A280 - and run a sample on a gel.