HFE PURIFICATION

Developed by Jose Lebron

Column Info:

Antibody:	anti-HFE (heavy chain) mAb 1C3.1G4
Bed volume:	3 - 4 ml
Wild-type HFE capacity:	approx. 3 mg
Flow rate:	0.75 ml/min max.
Made by:	Jose Lebron (3/96; for info see his notebook #6, p. 176)

Buffers:

		For 1 L:
A. Binding:	50 mM Tris, pH 7.4 0.05% NaAzide	50 ml of 1 M stock 5 ml of 10% stock 950 ml dH20
B. Elution:	50 mM DEA, pH 11 (DiEthylAmine)	For 250 ml: 1.29 ml DEA stock pH with concHCl to 10.9-11 (will create some vapor)

To neutralize fractions: 1 M monobasic NaPhosphate

Column run:

1. Run harvest over column

2. Wash with Buffer A for about 1 hr (about 40 ml)

3. Elute with Buffer B: collect 20 1 ml fractions (25 to elute tail completely). Add 100 ul 1 M monobasic $NaPO_4$ per tube before elution to neurtralize pH. Peak should begin to come off in fraction 8-11, depending on amount of liquid over column.

4. Wash with 20-30 ml Buffer A.

5. Eluted fractions: as long as pH is close to 7, protein may be stored in the DEA/phosphate buffer, but add NaCl to 0.1 M final.

Christina Milburn, 8/26/97