Preparation of KLH

KLH received from Pacific Biomarine, Venice, California

(received in $(NH_4)_2\ SO_4$ slurry)

1. Add an equal volume of 0.15 M NaCl and dialyze 24 hrs. against 2L 0.15 molar NaCl -2 changes of NaCl.
2. Spin at 10,000 r.p.m. in Sorvall for 15 min to remove precipitated protein,etc.
3. Spin supernatnat from previous spin in I.E.C. at 30,000 r.p.m. ~80,000g in A170 fixed angle rotor for 2 hrs to pellet protein.
4. ~~Resuspend pellet in 0.01M cacodylate (138 mg Cacodylate to 100 ml 0.15 M NaCl) and bring to pH 6.8 with conc. NaOH--do not go above pH 7.2 as KLH will start depolymerizing.~~ Determine protein conc.

$$OD_{280} \quad KLH\ E^{0.1\%}_{1\ cM} = 1.55$$

Store liquid at 4°C (no preservative addition).

resuspend pH 7.0 1x PBS

For fluoresceination of KLH, see FITC technique.

(Technique is personal communication from Pacific Biomarine)

Note · 4: resuspend in PBS.