Coupling of Phospho-ELKtide to KLH

1) Warm rotary for >1/2 hour at R.T.

2) Equilibrate PD10 column 5x5 ml with 1X PBS pH 7.

3) Dissolve 20 mg KLH/1 ml 1X PBS pH 7 in 1.5 ml eppendorf tube.

4) Dissolve 5 mg MBS (one vial) in 200 l dimethyl formamide under hood.
   Store on ice before use. Use within one hour.

5) Add 200 l MBS/DMF dropwise to KLH.
   React at R.T. for than 30' (no longer) on rotary.

6) During KLH/MBS rxn, set up the protein assay of PD10 elution:
   A. Mark tubes #1-10 for PD10 elution.
   B. Mark tubes #1-10 for Bradford Assay. Add to each tube:
      795 l H2O
      200 l Bradford reagents

7) Dissolve peptide in a scintillation vial with a small flea-stirbar:
   1 ml Phospho-ELK (5 mg/ml)
   60 l 0.5 M EDTA (20 mM final)
   440 l H2O
   Store on ice before use.

*Critical steps #8-9: perform as fast as possible!

8) At the end of 30’ incubation (step #5):
   Load the sample (~1.2 ml) to the pre-equilibrated PD10 column.
   Elute with 1X PBS, 0.5 ml at a time.
   Collect 0.5 ml fractions.

   Add 5 l eluate to protein assay tube; vortex.
   Combine fractions containing protein. (Should be in #5-7)
   Add 1X PBS to make 3 ml total volume; mix.
   Set aside 1ml for spec. readings.

9) Add 1.5 ml KLH/MBS to each peptide in scintillation vial.
   Bubble through with argon; close the vial.
   Stir at R.T. 4 hr.
   Set aside 100 ul for spec. readings.

10) Dialyze against cold PBS with 3-4 changes o/n: SpectrPor MW cut off 10,000.

11) After dialysis, add PBS to make up to 5 ml (peptide 1 mg/ml).
    Save 200 ul for spec. readings.
    Aliquot 350 l/tube => 14 tubes [350 ug peptide/rabbit for immunization]