

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 μ l dimethyl formamide under hood.
Store on ice before use. Use within one hour.
 - 5) Add 200 μ 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 μ l H₂O
 - 200 μ l Bradford reagents
 - 7) Dissolve peptide in a scintillation vial with a small flea-stirbar:
 - 1 ml Phospho-ELK (5 mg/ml)
 - 60 μ l 0.5 M EDTA (20 mM final)
 - 440 μ l H₂OStore 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 μ 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 μ l 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 μ l for spec. readings.
 - Aliquot 350 μ l/tube => 14 tubes [350 μ g peptide/rabbit for immunization]