

Preparation Of Peptide-KLH Conjugates For Immunization

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These are basically Tim's procedures and have been used successfully by numerous members of our lab and by others around us.

A. Checking Peptide Thiol Groups

Do this before thiol coupling, either to KLH or to resin. Peptide thiol groups have a tendency to get lost after synthesis.

1. Make up 5 mM Ellman's reagent (dithio-bis-2-nitrobenzoic acid) in 0.1M NaPi pH 7.2.
2. Weigh out about 1 mg of peptide into a tared tube.
3. Add 0.5 ml reagent. It should go bright yellow.
4. Dilute the mixture 1/50 in buffer. Read A412 against reagent at the same concentration.
5. Calculate the apparent molecular weight of the peptide based on thiol groups, using a molar extinction coefficient of 14,000. Compare this to the expected molecular weight of the peptide. They should agree within a factor of three, with the apparent molecular weight usually higher. If the thiol concentration is anomalously low, i. e., the apparent molecular weight is very high, there may be something wrong with the peptide- anyway it probably will not couple well. You may need to regenerate the thiol groups by reducing the peptide with excess DTT and running a P2 column.

B. Coupling of Peptide to KLH

This recipe is for two bunnies for about five injections per bunny

1. Weigh out 100mg of keyhole limpet hemocyanin (KLH). Dissolve in 2ml water. It generally takes about 4 hours to dissolve- you will need to sonicate and vortex. Be patient and put on a rotator at 4 deg C. Dialyze against 2l of 0.1M NaPhosphate pH 7.8 overnight. This is to remove any contaminating thiols or amino compounds.

2. Spin 10 minutes at full speed in microfuge to remove aggregates (don't be surprised to see a substantial pellet).
3. Split the KLH into 2 aliquots for -SH and -NH₂ coupling.
4. For -NH₂ coupling, add 5mg peptide to one aliquot, followed by glutaraldehyde to 0.1% final. Add the peptide as a solid if it is soluble, otherwise from a 100 mg/ml stock in DMSO. Precipitation does not matter and often happens. After adding the glut, check the pH with pH paper, and adjust to 7.8 if necessary using NaOH. Incubate 8-12 hrs at 4 degrees, rotating gently.
5. Add a **tiny** pinch of NaBH₄ to kill remaining glut. Make sure the sample is in a large tube since it tends to fizz up. Incubate 8-12 hrs at 4 degrees. This is the glut conjugate.
6. For the -SH coupling, warm the other aliquot of KLH to room temp. Add 1/9 th volume of Iodoacetic acid N-hydroxysuccinimide ester at 100mg/ml in DMSO. Make the DMSO stock fresh, and protect the iodoacetamide reagent from light. We make our own IAA-NHS ester, but it can be purchased from Sigma.
7. After 10 minutes at room temp the KLH will start to get a little cloudy. Load it onto a P-10 column equilibrated with 0.1M NaPhosphate pH 7.8. Make sure the column is at least 10 times the volume of the sample. Pool the KLH containing fractions by color (it will be sort of greyish green). Add 5mg of peptide to them, as in step 5 above. Incubate at least 8 hrs at 4 degrees, rotating gently.

Pool the coupled peptide from the two procedures. Dilute to 5ml with 0.15M NaCl. If there is a precipitate, sonicate vigorously to break it up. Split the immunogen into 1 ml aliquots (each aliquot will immunize two rabbits) and freeze it.