

Peptide conjugation to KLH for antibody production

1. Weigh out the appropriate amount of your peptide (typically 3-5 mg). Dissolve in PBS (Ca^{2+} and Mg^{2+} -free; Gibco catalog # 14190-144) to achieve a **10 mg/ml final concentration** (i.e. dissolve 3 mg of peptide in 0.3 ml of PBS or 5 mg in 0.5 ml PBS). If your peptide is not soluble in PBS, then try dissolving it in a small amount of DMSO (try 3-5 mg in 0.1-0.2 ml DMSO) and then diluting this further with PBS. If you do need to use DMSO, you should still try to achieve a 10 mg/ml final peptide concentration, and DMSO should be no more than 60% of this solution. For example if you are using 5 mg of peptide, you should dissolve it in no more than 0.3 ml of DMSO, and then add no less than 0.2 ml PBS to achieve a 5 mg/ml final concentration. Save your unused peptide at -20°C for future use in antibody production and/or affinity purification projects.
2. Reconstitute Imject Maleimide-conjugated KLH (Pierce catalog # 77605, can order through Fisher Scientific) as specified by the product insert. Typically, the 10 mg vial contains a bit more (11-12 mg), and you need to add an appropriate amount of dH₂O (not PBS- it has salts added to it already) to achieve a **10 mg/ml final concentration** (i.e. dissolve 11 mg of KLH in 1.1 ml of dH₂O or 12 mg of KLH in 1.2 ml of dH₂O). Mix by inverting the tube several times but do not vortex. The KLH will not actually dissolve but will instead form a blueish colored milky suspension. Vortexing may cause the KLH to fall out of suspension and form insoluble clumps.
3. Distribute the reconstituted KLH into eppendorf tubes according to the number of peptides that you are going to be using. If you are using 2 peptides, split the KLH into 0.5 ml amounts. If you are using 3 peptides, split the KLH into 0.35 ml amounts. You can store unused reconstituted KLH at -20°C for a short period of time, but I have not ever used this in my antibody production attempts. I prefer to use it all up where possible. KLH is expensive (~\$285 per vial), so try not to waste it.
4. Immediately add an equivalent volume of your 10 mg/ml peptide solution from step 1. For 0.5 ml of KLH add 0.5 ml of your peptide solution, and for 0.35 ml KLH add 0.35 ml of your peptide solution. This 1:1 mixture is referred to a 5 mg/ml KLH-conjugated peptide suspension in all following steps.
5. Mix the KLH/peptide suspension for 2 hours at room temperature on a Nutator.
6. Transfer the suspension into a dialysis cassette (such as a Pierce Slide-a-lyzer cassette (catalog # 66373) and dialyze overnight in PBS at 4°C.
7. Remove KLH-peptide solution from the dialysis cassette and transfer to an eppendorf tube. Freeze and ship out for antibody production.