Total approx. maleimide groups available in various carriers:
KLH (mol wt 4.5-x10^5-1x10^7 daltons); maleimide groups 150-300
BSA (mol wt 67000 daltons); maleimide groups 15-20
Ovalbumin (mol wt 45000 daltons); maleimide groups 5-15

Instructions for Use

1. Dissolve 2-5 mg of peptide in 1X conjugation buffer at 2 mg/ml. Adjust pH to 7 if necessary. **Note**: if the peptide is insoluble in water or aqueous solution, dissolve the peptides in DMSO (dimethylsulfoxide) first at 5-10 mg/ml and the dilute it with 1X conjugation buffer to reduce the DMSO to 20% or less (DMSO does not significantly alter the conjugation efficiency or solubility of the carrier proteins).

2. Take 1 vial (1 mg/200ul) of Pre-Activated carrier protein and add 0.3 ml of 1X conjugation Buffer.

3. Add peptide solution (2-5 mg in 1 ml) to the pre-activated carrier protein. Mix gently. Incubate at room temp. For 3-4 hours or at +4°C for 24 hours. The conjugated peptide is ready to use in an immunization protocol without any further purification. However, if the peptide is toxic or if DMSO was used then it is advisable to remove the DMSO and other additives by desalting on G-10 or P-30 column or by dialysis against PBS or 1X conjugation buffer for 10-12 hours at 4oC (use 100-500 mls PBS or conjugation buffer).

4. Store the conjugated peptide at -20oC or below in suitable size aliquots.

Typically, 100-200 ug of the conjugated peptide per rabbit/goat/chicken per injection etc can be used.

Peptide coupling efficiency measurements

The peptide conjugation to pre-activated proteins can be quantified by the following protocol:

1. Remove 1/10 volume of the conjugation reaction and place in a separate tube.
2. Add 50 µl of a 1 mg/ml solution of 5,5 Œ-dithio-*bis*-2-nitrobenzoic acid (Ellman's reagent, not supplied) in PBS (phosphate-buffered saline).
3. Incubate for 15 minutes at room temperature.
5. Measure the absorbance OD_{410}.
6. Determine the quantity of unconjugated peptide in the reaction cocktail by comparing the OD_{410} to a standard curve generated by 50, 100, 250, and 500 nM peptide with 50 µl of Ellman's reagent.
7. Subtract the remaining peptide from the initial peptide to determine the quantity of peptide conjugated to the BSA.
8. Determine the peptide:carrier ratio by dividing the nmoles of conjugated peptide by the quantity of carrier protein.

Instruction Manual No. M- 80240, 80250, 80260

Pre-activated BSA or KLH or Ovalbumin Kit

For Coupling 4 or more free Cysteine Containing peptides

- ☐ **Cat # 80240 (Preactivated-BSA)**
- ☐ **Cat # 80250 (Preactivated-KLH)**
- ☐ **Cat # 80260 (Preactivated-Ovalbumin)**

For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Introduction

All small peptides/haptens (such as steroids or drugs or chemicals) must be coupled to a carrier protein (KLH or Keyhole limpet hemocyanin, BSA, Bovine serum albumin or Ovalbumin, etc) in order to elicit high titer antibodies. Generally, peptides can be coupled to other proteins by utilizing:

1. **Free NH2** (recommended for peptide when Cysteine cannot be added to the peptides or not available or not desirable to modify it). Free NH2 group is available in all peptides at the N-terminus unless it has been blocked by acetylation or the addition of other groups such as biotin or FITC etc. Secondary amines available mostly by lysine (Lys or K) can also be utilized. Typically, activation of amines in the carrier proteins by glutaraldehyde and then coupling to the peptides or proteins provides good coupling.

2. F**ree COOH** (recommended for peptide when Cysteine cannot be added to the peptides or not available or not desirable to modify it). Free -COOH is available in all peptides at the C-terminus unless it has been blocked by amide or the addition of other groups such as biotin or FITC etc. Secondary -COOH available mostly by Aspartic acid (Asp or D) or Glutamic acid (Glu or E) can also be utilized. Typically, activation of –COOH in the carrier proteins by EDAC ((1-Ethyl-3-(3′-dimethylaminopropyl)carbodiimide)) and then coupling to the peptides or proteins provides good coupling.

3. **Free Cysteine (Cys)** group (available as part of the sequence or added at the N or C-terminus). Note: Any available free cysteine will be modified regardless of where it is. So there is no need to add a cysteine if there is one or more already available in the peptide.

The most important criteria in selecting the conjugation method are to do the minimal modification of the peptide as a result of the chemical conjugation. Cysteine is not as common as other amino acids that provide free amines (Lys, Arg etc) or free –COOH (Asp or Glu). In most cases, short peptides selected for antibody production may not have any cysteine, therefore, it is preferred to add a cysteine in the peptide sequence for synthesis and then conjugation. If the peptide is internal or it represents the very C-terminus then Cys should be added at the N-terminus. This will keep the COOH free (non-conjugated) as it exists in native protein. For peptides representing the very NH2-terminal sequences (starting from amino acid #1), Cys should be added at the C-terminus of the peptide. For internal peptides, Cys can be added at either end but it is easier to synthesize peptides containing a NH2-terminal Cysteine.

Activation of carrier proteins requires many chemicals and experience. It may be more expensive to buy all the chemicals and may take more time as well. Pre-activated carrier proteins (KLH, BSA or ovalbumin) available from ADI made the conjugation of the peptides very easy and quick.

Advantages of using pre-activated carrier proteins

- No need to buy individual reagents or deal with chemicals or know the peptide chemistry
- Kit contains all necessary reagents
- Conjugation is performed at room temp in ~3-4 hrs.

Kit Components

*__Pre-Activated BSA kit #80240__ contains 1 mg x 4 vials, # 80241
*__Pre-Activated KLH kit # 80250__ contains 1 mg x 4 vials, # 80251
*__Pre-Activated Ovalbumin kit # 80260__ contains 1 mg x 4 vials, # 80261

*Note: Store Pre-activated proteins (#80241, #80251, #80261) at -20oC upon arrival. Store Conjugation buffer CB-20 at 4oC.

Conjugation Buffer (20X), 25 ml; #CB-20 (common to kit# 80240, #80250, and 80260 kits). Dilute 1:20 with water. If buffer salts have forms crystals then they can be dissolved by warming or mixing at room temp for 1-2 hrs.

Pre-activated BSA/KLH Conjugation Chemistry

Carrier proteins (BSA, Ovalbumin or KLH etc) are preactivated using Sulfo-SMCC, a heterobifunctional cross-linker that contains a N-hydroxysuccinimide (NHS) ester and a maleimide group. After activation of the proteins, a reactive and stable Maleimides groups is available to react with free sulfydryl (Cys)-containing peptides or haptens. Maleimides react with sulfhydryls of the peptides at pH 6.5-7.5 to form stable thioether bonds. At pH values >7.5, reactivity toward primary amines and hydrolysis of the maleimide group can occur.

1. **Carrier**-NH2 +Sulfo-SMCC →(pH 7.5) ***Carrier**-**Maleimide** (Activate-Carrier)

2. *Carrier-**Maleimide** + **SH**-Peptide→(pH 7) Carrier- **Maleimide-S-peptide conjugate**