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A Geno Technology, Inc. (USA) brand name

ActiveHOOK™ KLH

(Cat. # 786-089)

INTRODUCTION

ActiveHOOK™ KLH is a pre-activated form of KLH (keyhole limpet hemocyanin) that has been activated by sulfoSMCC and is ready for direct coupling with peptides through the cysteine residues. KLH is isolated from the mollusc *Megathura crenulata* and belongs to a family of giant respiratory proteins, the hemocyanins, which are found in molluscs and arthropods. The large molecular weight of KLH (4.5×10^5 to 1.3×10^7 Daltons) makes it an excellent choice for an immunogen. ActiveHOOK™ KLH has a total of 300-600 reactive groups available for coupling and making ActiveHOOK™ KLH an outstanding carrier protein. ActiveHOOK™ KLH is supplied lyophilized as 10mg/vial.

ITEM(S) SUPPLIED (Cat. # 786-089)

Description	Size
ActiveHOOK™ KLH	10mg

STORAGE CONDITION

Shipped at ambient temperature. Upon receiving, store it at 4°C.

ADDITIONAL ITEMS REQUIRED

- **Peptide:** peptide of choice to be coupled.
- **Reaction Buffer**: Optimizer Buffer™ III (Cat. # BKC-06) or suitable buffer for sulfhydryl reactions.
- **Dialysis Equipment**: Tube-O-Dialyzer™ (MWCO 8kDa) (Cat. #786-617) or dialysis tubing (MWCO 8kDa).
- **Dialysis Buffer**: Suitable storage buffer, e.g. PBS.

IMPORTANT INFORMATION

1. *To ensure complete coupling, we advise using the recommended quantities of reagents, as these provide a molar excess of peptide to the maleimide groups on the carrier proteins.*
2. *For peptides insoluble in 1X Optimizer Buffer III, we recommend the use of DMSO (<30%).*
3. *The kit is designed for the coupling of protein and peptides to KLH through free sulfhydryl residues. Ellman's reagent (Cat #BC87) can determine the presence of free sulfhydryl groups. If oxidized, the proteins and peptides can be reduced with 0.1-0.5% β-mercaptoethanol (Cat #BC98), 5-50mM DTT (Cat #786-227) or 1-10mM TCEP (Cat #786-230). Following reduction, the reducing agent must be removed and we recommend using a gel*

filtration spin column (SpinOUT™, Ca. #786-170, 786-171). This method is not suitable for small peptides (MW < 1500).

PROTOCOL

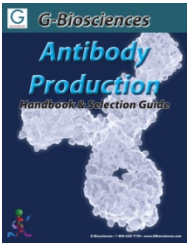
1. Aliquot 2mg *Active*HOOK™ KLH into suitable tube and dissolve it in 500µl 1X Optimizer Buffer™ III or suitable buffer for sulfhydryl reactions.
2. Immediately before use, dissolve 2-10mg sulfhydryl containing peptide or protein in 500µl 1X Optimizer Buffer™ III or suitable buffer for sulfhydryl reactions.
3. Add the sulfhydryl containing peptide to the ActiveHOOK™ KLH. Mix by inverting the tube 4-5 times.
4. Incubate at room temperature for 30 minutes. Mix the reaction and incubate for another 1 hour at 4°C.
5. Dialyze the reaction against a suitable storage buffer (e.g. PBS) for 1-2 hours, with 2-3 changes of dialysis buffer, to remove uncoupled peptides.
6. After dialysis, the carrier protein-peptide conjugate is ready for use. The carrier protein-peptide conjugate may be stored at -20°C for later use.

MODIFICATION OF –NH_2 TO –SH FOR PROTEINS AND PEPTIDES LACKING SULFHYDRYL GROUPS

1. Suspend the peptide or proteins to be thiolated in 50mM triethanolamine, 0.15M NaCl, 1mM EDTA pH8.0.
2. Add a 3-4 fold molar excess of Traut's reagent (Cat. # BC95).
3. Incubate the reaction for 45 minutes at room temperature, under nitrogen gas.
4. Separate the thiolated products from unreacted Traut's reagent, using a SpinOUT™ column (SpinOUT™ GT-600 (Cat. # 786-170)) or by dialysis.

RELATED PRODUCTS

Download our Antibody Production Handbook



http://info.gbiosciences.com/complete-Antibody-Production-handbook

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