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A Geno Technology, Inc. (USA) brand name

# HOOK™ Maleimide Activated Ovalbumin

For conjugation of Ovalbumin to sulfhydryl groups  
containing proteins, peptides and ligands

(Cat. #786-1655, 786-1656)



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## INTRODUCTION

Haptens are small molecules such as peptides that are not immunogenic but can bind to antibodies of appropriate specificity (hapten-specific antibodies). Although haptens are incomplete immunogens, they can be made fully immunogenic by coupling them with suitable carrier proteins. Some of the commonly used carrier proteins are keyhole limpet hemocyanin (KLH; MW:  $4.5 \times 10^5$  D), BSA (MW: 67,000D) and ovalbumin (OVA; MW: 45,000).

Ovalbumin (OVA) contains 20 lysines and is often used as secondary carrier in ELISA applications. OVA is a single polypeptide with half of its residues are hydrophobic. Ovalbumin is subject to denaturation from vigorous shaking and above 56°C.

G-Biosciences HOOK™ Maleimide Activated Ovalbumin is offered to enable its conjugation with hapten as a carrier protein for use in generating immune response or for ELISA applications.

Ovalbumin is maleimide activated using Sulfo-SMCC, a heterobifunctional crosslinker which adds a free maleimide group which reacts with sulfhydryl containing molecules.

HOOK™ Maleimide Activated Ovalbumin reacts with sulfhydryl containing peptides or haptens at pH 6.5-7.5 to form stable thioether bonds (Fig.1)

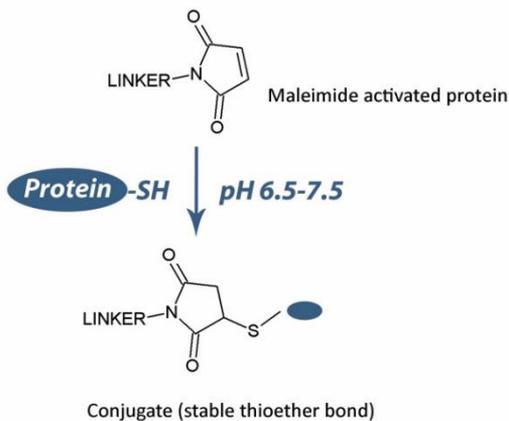


Fig1: Maleimide mediated conjugation reaction

## ITEMS SUPPLIED

Cat. #	Description	Size
786-1655	HOOK™ Maleimide Activated Ovalbumin	1 mg
786-1656	HOOK™ Maleimide Activated Ovalbumin	5 x 1 mg

## STORAGE CONDITIONS

HOOK™ Maleimide Activated Ovalbumin is supplied at ambient temperature. Upon receipt, store at -20°C under desiccating conditions.

## ADDITIONAL ITEMS NEEDED

1. Sulfhydryl group containing peptide or hapten to be conjugated.
2. Maleimide conjugation buffer [Optimizer Buffer™ III (5X), Cat. # BKC-06] or 100 mM sodium phosphate 5-10 mM EDTA, pH7.6.

## IMPORTANT INFORMATION

1. Reconstitute the HOOK™ Maleimide Activated Ovalbumin immediately before the conjugation reaction. Maleimide groups in solution are hydrolyzed and become non-reactive, no stock solutions for storage should be made. Any left-over solution should be discarded.
2. Sulfhydryl containing compounds should be avoided during conjugation reaction as these will react with maleimide groups and reduce the conjugation efficiency with the desired molecule (Table 1).
3. The conjugation reaction for maleimide activated proteins should be at pH 6.5 to 7.5, where it forms stable thioether bonds. Maleimide groups can hydrolyze or show reactivity toward primary amines at pH greater than 7.5.
4. In general, 1 mg HOOK™ Maleimide Activated Ovalbumin to label 1 mg of peptide or hapten solution. However, if optimum concentration is necessary for more efficient results, one can optimize hapten molecule to HOOK™ Maleimide Activated Ovalbumin ratio.

Interfering agents	Recommended
pH	Neutral (6.5-7.5)
Primary amines	Yes
Reducing reagents	<b>NO</b>
Sodium azide	<0.1%

**Table 1:** Recommended buffer conditions and components

## PROTOCOL

### **Preparation of hapten for conjugation to maleimide activated ovalbumin**

1. Peptides to be conjugated must have free sulfhydryl groups. Sulfhydryl groups can be introduced to protein using SATA or Traut's Reagent (Cat. # 786-1645, 786-1650).

2. Peptides should be dissolved in maleimide conjugation buffer. If peptide is present in buffer with pH > 7.5 or contains reducing agents, it should be dialyzed (Tube-O-DIALYZER™, Cat. #786-610 to 786-624) against the maleimide conjugation buffer or use desalting column (SpinOUT™ GT-600, Cat. # 786-704 or SpinOUT™ G-Acryl 600, 5ml, Cat. # 786-1623) for buffer exchange.

### **Conjugation reaction**

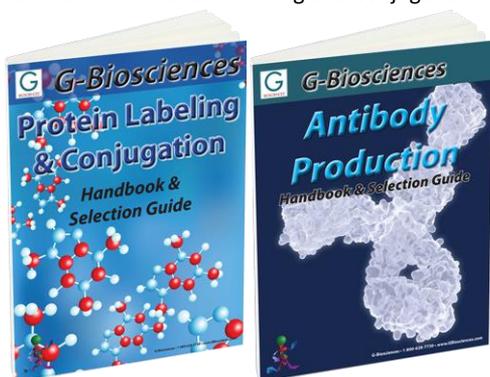
1. Allow the vial of HOOK™ Maleimide Activated Ovalbumin to warm to room temperature.
2. Remove the crimp seal and add the protein solution to the lyophilized powder.
3. Dissolve the lyophilized HOOK™ Maleimide Activated Ovalbumin in protein solution with the help of a pipette.
4. Incubate the solution for 3-4 hours at room temperature. Alternatively, the conjugation can be set overnight at room temperature.
5. For use of conjugate as immunogen, EDTA present in the conjugate buffer should be removed either by dialysis (Tube-O-DIALYZER™, Cat. #786-610 -786-624) or gel filtration (Spin-OUT™ GT-600, Cat. # 786-704 or SpinOUT™ G-Acryl 600, 5ml, Cat. # 786-1623) against PBS buffer.
6. Store the conjugated protein at 4°C.

### **STORAGE OF CONJUGATED ANTIBODIES/PROTEINS**

Store the ovalbumin conjugated hapten molecules at 4°C. Conjugates can be stored at -20°C after adding glycerol up to 50% concentration. Optimum storage for a conjugate should be determined by experimentation.

### **RELATED PRODUCTS**

Download our Protein Labeling and Conjugation and Antibody Production Handbooks.



<http://info2.gbiosciences.com/complete-protein-labeling-conjugation-handbook>

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