

## Introduction

Activated KLH, BSA and Ovalbumin (OVA), are commonly used as protein carriers for haptens such as peptides in order to enable the immune response to small molecules. In many cases KLH conjugate is used for the immunization, while BSA or OVA conjugate is used in immunoassays of the resulting antibodies. OVA conjugates are used in order to filter out the antibodies directed to the KLH. However in some cases OVA or other proteins are preferred as carrier proteins because of a variety of other reasons.

The Maleimide activated KLH and OVA produced by Adar Biotech are preactivated with a heterobifunctional cross-linker (GMBS). These activated proteins may be reacted with biomolecules that contain a free sulfhydryl groups, to form a stable thioether bond.

## Maleimide-Activated OVA characteristics.

Activation method: GMBS.

Binding capacity: ~2-4 mg of peptide (average MW of 1000-2500) per 2 mg OVA

Protein concentration: 4 mg/ml (0.5 ml)

Storage buffer: PBS pH 7.5

Transportation conditions: Ambient temperature up to 7 days.

Storage condition: -20°C.

## A. Procedure for Peptide Conjugation

1. Dissolve the sulfhydryl-containing hapten in a volume of water (or suitable buffer) equals to x0.4 to x1 times the volume of OVA. For example dissolve 2 mg of peptide in 200-500 µl of buffer for addition to 2 mg of activated OVA in 500 µl.

**Note:** For haptens with limited solubility, DMSO may be used for solubilization. Use .30% DMSO in the final conjugation solution or the carrier protein may irreversibly denature.

Alkaline pH values (above 8.5) may hydrolyze the maleimide group or generate side reactions with amines. Haptens must contain cysteine or a sulfhydryl group in the reduced state in order to react efficiently with the maleimide group.

2. Thaw the Maleimide Activated OVA at room temperature.

**Note:** Do not vortex or heat the activated OVA.

3. Mix the peptide with activated OVA adding peptide stepwise (1/4<sup>th</sup> of quantity each time in 5 minutes intervals) and watching for possible appearance of turbidity in solution. If slight turbidity forms – stop adding peptide to KLH solution. Reaction is done for 2 hours at room temperature under occasional tapping of tube.

4. Peptide-conjugated OVA can be purified by gel filtration or dialysis to remove unbound peptide

Note: If the immunogen is to be stored for more than a few days it is recommended to store frozen at -20°C.

5. The coupling efficiency of conjugation can be determined by assaying the content of free sulfhydryl groups in the unreacted peptide using DTNB reagent.

## **B. Storage**

The activated-OVA should be stored frozen until use.