



INSTRUCTIONS

Maleimide-Activated OVA

(Cat. 1026-2/10)

ver. 1.0, 6.7.05

Introduction

Activated KLH and BSA 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 immunization while BSA conjugate is used in immunoassays of the resulting antibodies in order to filter out the anti KLH response. However, in some cases BSA or Ovalbumin are preferred as carrier proteins for immunizations.

The Maleimide activated OVA produced by Adar Biotech is preactivated with a heterobifunctional cross-linker (GMBS). The activated protein can be reacted with compounds that contain a free sulfhydryl group 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

Storage condition: -20°C.

A. Procedure for Peptide Conjugation

1. Dissolve the sulfhydryl-containing hapten in a volume of water equal to 1.0-2.5 times the volume of the activated OVA. For example dissolve 2 mg of peptide in 200-500 μ l of buffer for addition to 2 mg of activated OVA in 500 μ l.

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. Immediately mix the peptide and activated mcOVA and react for 2 hours at room temperature.

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

Note: If the immunogen is to be stored for more than a few days it is recommended to store in a 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.