

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.

The Maleimide activated BSA produced by Adar Biotech are 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 BSA characteristics.

Activation method: GMBS.

Binding capacity: ~2.5-4 mg of peptide (average MW of 1000-2500) per 2 mg BSA

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 BSA. For example dissolve 2 mg of peptide in 200-500 µl of buffer for addition to 2 mg of activated BSA 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 BSA at room temperature.

Note: Do not vortex or heat the activated BSA.

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

4. Peptide-conjugated BSA 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-BSA should be stored frozen until use.