INSTRUCTIONS

Maleimide-Activated BSA

(Cat. 1025-2/10)

ver. 1.1, 03.07

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 the immunization, while BSA conjugate is used in immunoassys of the resulting antibodies. BSA conjugates are used in order to filter out the antibodies directed to the KLH. However in some cases BSA or other proteins are preffered as carrier proteins because of a variety of other reasons.
The Maleimide activated KLH and BSA 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 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 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 BSA and react for 2 hours at room temperature.

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

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