ADAR BIOTECH

INSTRUCTIONS

Maleimide-Activated KLH

(Cat. 1027-2/10)

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. KLH is most popular for immunization while BSA conjugate is used in immunoassays of the resulting antibodies in order to filter out the anti KLH response.
The Maleimide activated KLH or BSA produced by Adar Biotech are preactivated with a heterobifunctional cross-linker (GMBS). These activated proteins can be reacted with compounds that contain a free sulfhydryl group to form a stable thioether bond.

Maleimide-Activated KLH characteristics.

Activation method: GMBS.
Binding capacity: ~2-4 mg of peptide (average MW of 1000-2500) per 2 mg KLH
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 KLH. For example dissolve 2 mg of peptide in 200-500 µl of buffer for addition to 2 mg of activated KLH 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 KLH at room temperature.

Note: Do not vortex or heat the activated KLH.

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

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

.