Maleimide Activated BSA-Peptide Conjugation Kit

05/21

(Catalog # K2090-5; 5 Columns; Store at -20 °C)

I. Introduction:

Bovine Serum Albumin (BSA) is a commonly used carrier protein in antibody production. The haptens, small molecules or peptides, are normally conjugated to BSA for enhancing their immune response. Maleimide Activated BSA is a popular form of BSA for making hapten/antigen-BSA conjugates. **BioVision's Maleimide Activated BSA-Peptide Conjugation Kit** is a fast and convenient tool for preparing conjugates of sulfhydryl-containing haptens and BSA carrier protein. The kit contains all the necessary reagents including the Maleimide Activated BSA carrier protein, buffers and columns for purifying the peptide hapten-carrier protein conjugates from the unreacted hapten.

II. Application:

- Conjugation of sulfhydryl-containing hapten to Maleimide Activated BSA for strong immune response.

III. Sample Type:

- Cysteine containing peptides or other sulfhydryl-containing small molecules

IV. Kit Contents:

Components	K2090-5	Cap Code	Part Number
Maleimide Activated BSA	2 mg x 5	NM	K2090-5-1
Conjugation Buffer	3 ml	NM	K2090-5-2
Purification Buffer	10 ml x 5	NM	K2090-5-3
Desalting Column	5	--	K2090-5-4

V. User Supplied Reagents and Equipment:

- Sulfhydryl-containing hapten
- Centrifuge
- 15 ml conical tubes
- PBS (optional)
- DMSO (optional)

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C. Once the kit is opened, store Maleimide Activated BSA at -20 °C. Store all other reagents at 4 °C.

VII. Peptide Conjugation Protocol:

1. Conjugation:

a) Dissolve the sulfhydryl-containing hapten in Conjugation Buffer (2 mg in 200 µl of Conjugation Buffer). **Note: If the hapten is insoluble in aqueous solution, follow the alternative protocol below:**

Dissolve the sulfhydryl-containing hapten in DMSO (2 mg in 40 µl DMSO). Then add 160 µl Conjugation Buffer.

b) Reconstitute one 2 mg vial of Maleimide Activated BSA in 200 µl of ddH_2O at room temperature (RT) to generate Maleimide Activated BSA solution at 10 mg/ml, immediately before conjugation.

c) Add hapten (step 1a) to the reconstituted Maleimide Activated BSA (step 1b) and mix well.

d) Incubate for two hours at RT with gentle shaking. **Note: Carry out step 2 after 1 hour of incubation.**

2. Column Preparation:

a) Add 4 ml of ddH_2O, replace the cap and let the beads swell in the Desalting Column at RT. Mix well for 30 min.

b) Remove the bottom closure and loosen the cap.

c) Place the column in a 15 ml centrifuge tube and centrifuge at 1000 x *g* for 2 min. Discard the flow through.

d) Wash the column with 3 ml of ddH_2O. Centrifuge at 1000 x *g*, 2 min. Repeat 5 times.

e) Equilibrate the swelled column (from step 2a) with 2 ml Purification Buffer. Centrifuge at 1000 x *g* for 2 min. Repeat 3 times

3. Purification:

a) Place the column into a clean 15-mL conical tube. Add the hapten-BSA conjugate solution (from step 1c) to the column. **Note: Do not disturb the column bed.**

b) Centrifuge at 1000 x *g* for 2 min and collect the filtrate for immunization.

c) If the samples are to be used after more than 3 days post collection, store them in sterile tubes at -20 °C. **Note: Perform all the steps in a clean, sterile environment.**

VIII. Related Products:

- Hemocyanin-Keyhole Limpet (KLH) subunits, powder (Cat# 6286-1)
- Hemocyanin-Keyhole Limpet (KLH) subunits, solution (Cat# 6287-20)
- Hemocyanin-Keyhole Limpet (KLH), Native (Cat# 6288-25)
- Maleimide activated KLH Peptide Conjugation Kit (Cat# 2039-5)
- Maleimide Activated KLH (Cat# M1317-2,-10)
- Maleimide Activated BSA (Cat# M1316-2,-10)
- Maleimide Activated OVA (Cat# M1318-2,-10)

FOR RESEARCH USE ONLY! Not to be used on humans.