



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:

- 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.

- Reconstitute one 2 mg vial of Maleimide Activated BSA in 200 µl of ddH₂O at room temperature (RT) to generate Maleimide Activated BSA solution at 10 mg/ml, immediately before conjugation.
- Add hapten (step 1a) to the reconstituted Maleimide Activated BSA (step 1b) and mix well.
- Incubate for two hours at RT with gentle shaking. **Note: Carry out step 2 after 1 hour of incubation.**

2. Column Preparation:

- Add 4 ml of ddH₂O, replace the cap and let the beads swell in the Desalting Column at RT. Mix well for 30 min.
- Remove the bottom closure and loosen the cap.
- Place the column in a 15 ml centrifuge tube and centrifuge at 1000 x g for 2 min. Discard the flow through.
- Wash the column with 3 ml of ddH₂O. Centrifuge at 1000 x g, 2 min. Repeat 5 times.
- 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:

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