



# Maleimide Activated KLH-Peptide Conjugation Kit

rev 05/21

(Catalog # K2039-5; 5 Columns; Store at -20 °C)

## I. Introduction:

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

## II. Application:

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

## III. Sample Type:

- Cysteine containing peptides or other sulfhydryl-containing small molecules

## IV. Kit Contents:

Components	K2039-5	Cap Code	Part Number
Maleimide Activated KLH	2 mg x 5	NM	K2039-5-1
Conjugation Buffer	3 ml	Amber/NM	K2039-5-2
Purification Buffer	10 ml x 5	NM	K2039-5-3
Desalting Column	5	-	K2039-5-4

## V. User Supplied Reagents and Equipment:

- Sulfhydryl-containing hapten
- Centrifuge
- PBS (optional)
- DMSO (optional)

## VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C. Once the kit is opened, store Maleimide Activated KLH at -20 °C. Once opened, 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). Add 160 µl Conjugation Buffer.

b) Reconstitute one 2 mg vial of Maleimide Activated KLH in 200 µl of ddH<sub>2</sub>O at room temperature (RT) to generate Maleimide Activated KLH solution at 10 mg/ml immediately before conjugation.

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

d) Incubate for 2 hours at RT with gentle shaking.

### 2. Column Preparation:

a) Add 4 ml of ddH<sub>2</sub>O, 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<sub>2</sub>O. Centrifuge at 1000x 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:

b) Place the column into a sterile 15-mL conical tube (not supplied). Add the hapten-KLH 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 keep the sample for immunization.

d) If the samples are to be used after more than 3 days, store it in sterile tubes at -20 °C. **Note: Perform all 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 (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.**