CREATIVE BIOMART®
Assay Kit

KLH Conjugation Kit

Product Information

Common Name

KLH

Cat.No. Kit-2086

Product Overview

The KLH Conjugation kit is one-step conjugation of a hapten to a carrier protein using the carboxylreactive carbodiimide as the crosslinker. The resulting conjugate is used for eliciting an immune response and antibody production against the hapten. The carboxyl-reactive carbodiimide reacts with exposed carboxyl and amino groups on peptides and proteins to form stable bonds. These kits contain mcKLH formulated in buffers compatible with the carboxylreactive carbodiimide reactions and desalt spin columns, which offer exceptional protein recovery by simple centrifugation step.

Description

Keyhole Limpet Hemocyanin (KLH) is one of the most commonly used carriers in the conjugation of peptides for antibody production. Mariculture keyhole limpet hemocyanin (mcKLH) is a hemocyanin from the Concholepas concholepas mollusk with immunogenic properties similar to KLH but is more stable and efficient as a carrier protein for the production of antibodies to haptens and peptides. It contains numerous sites per molecule for effective conjugation of peptides and other antigens using amine-reactive or carboxyl-reactive crosslinkers. mcKLH is currently the industry standard for antibody production against a hapten or peptide. This KLH Conjugation kit is primarily optimized for the simple preparation of hapten-carrier conjugates for immunization and antibody production.

Synonyms

Keyhole Limpet Hemocyanin Conjugation Kit

Size

1 kit

Kit Components

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A. mcKLH: 2 × 2mg; Freeze (<-15 °C), Minimize light exposure
B. Conjugation Buffer (pH 4.7): 20 mL; Refrigerate (2-4 °C), Minimize light exposure
C. EDC (1-ethy 1-3-[dimethylaminopropyl] carbodiimide hydrochloride): 2 × 10 mg; Refrigerate (2-4 °C), Minimize light exposure
D. Purification Buffer Salts (pH 7.2): 2 bottles (10 mL/bottle); Freeze (<-15 °C)
E. Spin Desalting Columns (7K MWCO): 2 × 2 mL; Refrigerate (2-4 °C)

Assay Protocol

1. Prepare KLH-Hapten Conjugation:
1.1 Add 200 uL of ddH2O into the vial of mcKLH (Component A) to make a 10 mg/mL solution.
Note: mcKLH solution appears translucent to whitish-blue typically. Do not vortex or heat the solution, which will precipitate the carrier.
1.2 Hapten solution: Dissolve up to 2 mg hapten in 450 uL Conjugation Buffer (Component B).
Note: Some haptens might have limited solubility, use DMSO (< 30% in the final conjugation solution) to dissolve it first. Higher concentration of DMSO might irreversibly denature the carrier protein.
1.3 Add the 450 uL hapten solution (from Step 1.2) into the 200 uL of mcKLH solution (from Step 1.1) to have KLH Hapten working solution.
1.4 Dissolve one vial of EDC (Component C, 10 mg) in 1 ml of ddH2O and immediately add 50 µl of this solution to the KLH-Hapten solution (from Step 1.3), mix it gently. Incubate at room temperature for 2 hours.
1.5 Purify the conjugate by desalting to remove non-reacted crosslinker and protein preservative (e.g., sodium azide).
2. Purify BSA-Hepten conjugate:
2.1 Twist off the bottom closure of the desalting column (Component E), and loosen the cap. Place the column in a collection tube.
2.2 Centrifuge the column at 1,000g for 2 minutes to remove the storage solution.
2.3 Remove the cap and slowly add 1 mL of purification buffer to the column. Centrifuge at 1,000g for 2 minutes, remove the buffer. Repeat this step for 3 additional times, discarding the buffer from the collection tube.
2.4 Place the column to a new collection tube, and gently apply the sample into the center of the

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compact resin bed.
2.5 Centrifuge the column at 1,000g for 2 minutes to collect the sample.
2.6 The KLH-Hapten conjugate can now be used for immunization. If the KLH-Hapten conjugate is to be stored for more than a few days, sterile filter the conjugate, and store at 4 °C or - 20 °C.
Note 1: If the conjugate is to be used within one week, PBS may be used for purification. If the conjugate will be frozen, use the purification buffer salts (Component D) for purification.
Note 2: If DMSO is used in the conjugation, prepare the purification buffer salts with the same percentage of DMSO used for conjugation. This will minimize the precipitation in the column during desalting.
Note 3: If a precipitate formed during conjugation, centrifuge the precipitated material, collect the supernatant and save the precipitate. Purify the supernatant. Combine the precipitate and the purified conjugate.

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