ReadiLink™ KLH Conjugation Kit

Catalog number: 5502
Unit size: 2 Reactions

Component	Storage	Amount
Component A: mcKLH	Freeze (< -15 °C), Minimize light exposure	2 X 2 mg
Component B: Conjugation Buffer (pH 4.7)	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (20 mL)
Component C: EDC (1-ethy 1-3-[dimethylaminopropyl] carbodiimide hydrochloride)	Refrigerated (2-8 °C), Minimize light exposure	2 X 10 mg
Component D: Purification Buffer Salts (pH 7.2)	Freeze (< -15 °C)	2 X 10 mL
Component E: Spin Desalting Columns (7K MWCO)	Refrigerated (2-8 °C)	2 X 2 mL

OVERVIEW

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 ReadiLink™ KLH Conjugation kit is primarily optimized for the simple preparation of hapten-carrier conjugates for immunization and antibody production. The ReadiLink™ KLH Conjugation kit is one-step conjugation of a hapten to a carrier protein using the carboxyl-reactive 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 carboxyl-reactive carbodiimide reactions and desalt spin columns, which offer exceptional protein recovery by simple centrifugation step.

AT A GLANCE

Protocol Summary

1. Prepare protein solution
2. Prepare hapten solution
3. Mix protein with hapten into EDC
4. Incubate the reaction at RT for 2 hrs
5. Purify the conjugate by desalting

Important
The following protocol is a general protocol for a wide variety of haptens. Optimize the protocol accordingly for the conjugation efficiencies upon the size and structure of your hapten. Using a molar excess of hapten over carrier protein ensures efficient conjugation. In general, a reaction with equal mass amounts of hapten and carrier protein will achieve sufficient molar excess.

PREPARATION OF WORKING SOLUTION

1. mcKLH solution (10 mg/mL)
Add 200 µL of ddH_2O 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.

2. Hapten solution
Dissolve up to 2 mg hapten in 450 µL 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.

3. KLH-Hapten working solution

Add the 450 µL hapten solution into the 200 µL of mcKLH solution to have KLH-Hapten working solution.

SAMPLE EXPERIMENTAL PROTOCOL

KLH-Hapten conjugation

1. Dissolve one vial of EDC (Component C, 10 mg) in 1 mL of ddH_2O and immediately add 50 µL of this solution to the KLH-Hapten working solution, mix gently. Incubate at room temperature for 2 hours. Purify the conjugate by desalting to remove non-reacted crosslinker and protein preservative (e.g., sodium azide).

2. Twist off the bottom closure of the desalting column (Component E), and loosen the cap. Place the column in a collection tube.

3. Centrifuge the column at 1,000g for 2 minutes to remove the storage solution.

4. 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.

5. Place the column to a new collection tube, and gently apply the sample into the center of the compact resin bed.

6. Centrifuge the column at 1,000g for 2 minutes to collect the sample.

7. 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 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. 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. 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.

EXAMPLE DATA ANALYSIS AND FIGURES

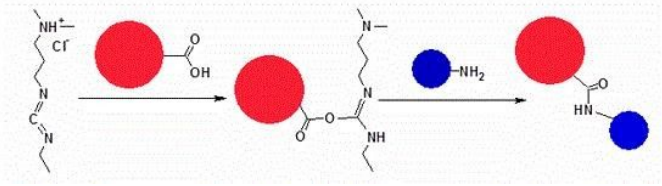

Figure 1.
EDC reacts with a carboxyl group of carrier protein BSA or KLH (represented by the red ball), forming an amine-reactive O-acylisourea intermediate (the central

molecule). The O-acylisourea intermediate reacts with an amine group on the antigen molecule represented by the smaller blue ball, yielding a conjugate of the two molecules joined by a stable amide bond [Please note the O-acylisourea inermediate is also susceptible to hydrolysis, making it unstable and short-lived in aqueous solution].

DISCLAIMER

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