ReadiLink™ KLH Conjugation Kit

Catalog number: 5502, 5502-10MG
Unit size: 2 Reactions, 2 Reactions (Bulk)

Component	Storage	Amount (Cat No. 5502)	Amount (Cat No. 5502-10MG)
Component A: mcKLH	Minimize light exposure, Refrigerated (2-8 °C)	2 X 2 mg	2 x 10mg
Component B: Conjugation Buffer (pH 4.7)	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (20 mL)	One bottle (20 mL)
Component C: EDC (1-ethy 1-3-[dimethylaminopropyl] carbodiimide hydrochloride)	Refrigerated (2-8 °C), Minimize light exposure	2 X 10 mg	2 x 10 mg
Component D: Purification Buffer Salts (pH 7.2)	Refrigerated (2-8 °C)	2 X 10 mL	One bottle (50 mL)
Component E: Spin Desalting Columns (7K MWCO)	Refrigerated (2-8 °C)	2 X 2 mL	2 x 10 mL

OVERVIEW

ReadiLink™ KLH Conjugation Kit is designed for the preparation of hapten-carrier and peptide-carrier conjugates for immunization and antibody production. Keyhole limpet hemocyanin (KLH) and related hemocyanin carrier proteins are commonly used in antibody production because their large size and strong immunogenicity help enhance immune responses against small antigens such as haptens and peptides. The kit uses mcKLH, a hemocyanin carrier protein formulated in buffers compatible with carbodiimide-mediated conjugation chemistry. The carboxyl-reactive carbodiimide activates carboxyl groups on peptides, haptens, or proteins, allowing coupling to available amino groups to form stable amide bonds. ReadiLink™ KLH Conjugation Kit also includes desalting spin columns for convenient cleanup and recovery of the carrier conjugate. The resulting hapten- or peptide-carrier conjugates are suitable for immunization workflows and antibody production against small antigens that are poorly immunogenic on their own.

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 Note

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

mcKLH solution (10 mg/mL)

Add 200 µL (for cat. #5502) or 1 mL (for Cat. #5502-10mg) 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.

Hapten solution

For cat. #5502, dissolve up to 2 mg hapten in 450 µL Conjugation Buffer (Component B). For cat. #5502-10mg, dissolve up to 10 mg hapten in 2 mL Conjugation Buffer (Component B).

Note: Some haptens might have limited solubility, use DMSO (≤30% in the final conjugation solution) to dissolve it first. Higher concentrations of DMSO might irreversibly denature the carrier protein.

KLH-Hapten working solution

For cat. #5502, add the 450 µL hapten solution into the 200 µL of mcKLH solution to have KLH-Hapten working solution. For cat. #5502-10mg, add the 2 mL hapten solution into the 1mL 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 (for cat. #5502) or 250 µL (for cat. #5502-10mg) 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 (do not remove the cap). Place the column in a collection tube.
3. For cat. # 5502, centrifuge the column at 1,000g for 2 minutes to remove the storage solution.
   For cat. #5502-10mg, place the column into a 50 ml conical tubes collection tube and centrifuge at 1000g for 5 minutes to remove the storage solution.
   Discard flow through and replace the column back into the collection tube.
4. For cat. #5502, remove the cap and slowly add 1 mL of purification buffer to the column. Centrifuge at 1000g for 2 minutes, remove the buffer. Repeat this step for 3 additional times, discarding the buffer from the collection tube.
   For cat. #5502-10mg, remove the cap and slowly add 5 mL purification buffer to the column. Centrifuge at 1000g for 5 minutes, discarding the flow through, and replace the column back into the collection tube. Repeat this step 3 times.
   Note: After each spin, the resin should appear white and free of liquid. If liquid is present, ensure the correct centrifugation speed and time. Incomplete centrifugation can result in poor sample recovery or sample dilution.
5. Blot the bottom of the column to remove excess liquid. Place the column to a new collection tube, and gently apply the sample into the center of the compact resin bed.
6. For cat. #5502, centrifuge the column at 1000g for 2 minutes to

collect the sample.
For cat. #5502-10mg, centrifuge the column at 1000g for 5 minutes and collect the flow through that contains sample. Discard the spin column.

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

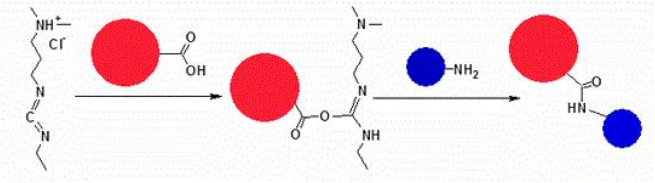

Figure 1. EDC reacts with a carboxyl group of carrier protein BSA or KLH (represented by the red ball), forming an amine-reactive O-acylisourea intermediate (the central molecule). The O-acylisourea intermediate reacts with an amine group on the antigen molecule represented by the smaller blue ball,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