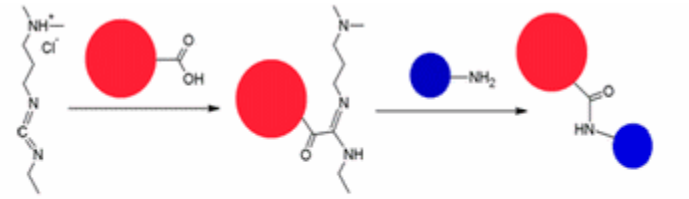
ReadiLink™ KLH Conjugation kit *For Antibody Development*

Ordering Information:	*Storage Conditions:*
Product Number: 5502	Multiple storage conditions

Introduction

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 cross linkers. 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 cross linker. 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. *You must make sure you hapten molecule has a free caroxy group to use this kit.*



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.

Kit Components

Components	Amount	Handling and Storage
A. mcKLH	2 × 2mg	Store at ≤ -20°C
B. Conjugation buffer (pH 4.7)	20 mL	Store at 4 °C
C. EDC (1-ethy 1-3-[dimethylaminopropyl] carbodiimide hydrochloride)	2 × 10 mg	Store at 4 °C
D. Purification buffer (pH 7.2)	2 x 10 mL	Store at ≤ -20°C
E. Spin Desalting Columns (7K MWCO)	2 × 2 mL	Store at 4 °C *Do not freeze*

Conjugation Protocol

Brief Summary

Prepare protein solution → Prepare hapten solution→ Mix protein with hapten into EDC → Incubate the reaction at RT for 2 hr→ Purify the conjugate by desalting

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.

1. Prepare KLH-Hapten Conjugation:

1.1 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.
1.2 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.
1.3 Add the 450 µL hapten solution (from Step 1.2) into the 200 µL of mcKLH solution (from Step 1.1) to have KLH-Hapten mixture solution.
1.4 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 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 cross linker and protein preservative (e.g., sodium azide).

2. Purify KLH-Hapten conjugate:

2.1 Thaw 1 bottle of Purification Buffer (Component D) to room temperature before use. Unused buffer can be stored at 4 °C for 1 week.
2.2 Twist off the bottom closure of the desalting column (Component E), and loosen the cap. Place the column in a collection tube.
2.3 Centrifuge the column at 1,000g for 2 minutes to remove the storage solution.
2.4 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.
2.5 Place the column to a new collection tube, and gently apply the sample into the center of the compact resin bed.
2.6 Centrifuge the column at 1,000g for 2 minutes to collect the sample.
2.7 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 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.

References

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2. Enomoto H, Li CP, Morizane K, Ibrahim HR, Sugimoto Y, Ohki S, Ohtomo H, Aoki T. (2008) Improvement of functional properties of bovine serum albumin through phosphorylation by dry-heating in the presence of pyrophosphate. J Food Sci, 73, C84.
3. Ledesma-Osuna AI, Ramos-Clamont G, Vazquez-Moreno L. (2008) Characterization of bovine serum albumin glycated with glucose, galactose and lactose. Acta Biochim Pol, 55, 491.
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