YamayBio

Native SHC Protein (Shrimp Hemocyanin, Non-Activated, KLH Substitution)

QUICK START GUIDE

For research use only.
Not for use in diagnostic procedures.

Contents and Storage

Native SHC Protein (Shrimp Hemocyanin, Non-Activated, KLH Substitution)

SH2170-10 mg

SH2170-100 mg

SH2170-100 mg × 6

Storage: Store at -20 °C. The product is shipped at room temperature.

Introduction

YamayBio Mariculture SHC is a purified shrimp (*Penaeus vannamei*) hemocyanin. It can be used as a substitute for Keyhole Limpet Hemocyanin (KLH) as a carrier protein for conjugation with low-molecular-weight molecules, such as peptides, nucleic acids, drugs, or toxins, thereby enhancing their immunogenicity. Comparative testing has shown that SHC exhibits immunogenicity comparable to KLH, while offering significantly improved solubility, particularly after hapten conjugation, providing greater flexibility in immunogen preparation protocols.

Molecular Weight: 70 kDa and 73 kDa

Appearance: Dark blue powder

Purity (MPLC-SEC): ≥ 98%

Extinction Coefficient: 280 nm(ε=1.277 $cm^{-1}\times mg^{1}\times mL$)

Native PAGE analysis: Two main characteristic bands close to 70 kDa (MW standard)

Endotoxin Level: ≤11.7 USP-EU/mg

Product Form: Lyophilized powder. Reconstitute with ultrapure water before use.

Procedure for Conjugation SHC to Peptides

SMCC-mediated conjugation

SMCC Activation

1. Dissolve the lyophilized SHC powder in an appropriate amount of ultrapure water to obtain a 10 mg/mL SHC solution.
2. Add 2 mL of freshly prepared SMCC solution (5 mg/mL in ultrapure water) to 2 mL of the reconstituted SHC solution.
3. Incubate at room temperature for 60 minutes or at 37 °C for 30 minutes, gently mixing at regular intervals.
4. Remove excess SMCC using molecular sieve chromatography.

Conjugation with Hapten:

1. Dissolve 20 mg of thiol-containing hapten in 5 mL of coupling buffer (83 mM sodium phosphate, 0.1 M EDTA, 0.9 M NaCl, 0.1 mM TCEP, pH 7.2).
2. Immediately mix the hapten solution with the activated SHC and incubate at room temperature for 2 hours. Remove excess SMCC using molecular sieve chromatography.
3. Remove EDTA using molecular sieve chromatography.

Note: The addition of 0.1 mM TCEP is optional and may be used to reduce disulfide bonds in the hapten to free thiol groups prior to conjugation.

EDC-Mediated Conjugation

EDC Activation

1. Equilibrate EDC and NHS to room temperature before use.
2. Dissolve the lyophilized SHC powder in an appropriate amount of activation buffer (0.1 M MES, 0.5 M NaCl, pH 6.0) to obtain a 1 mg/mL SHC solution.
3. Add 0.4 mg of EDC and 0.6 mg of NHS to 1 mL of the SHC solution, mix thoroughly, and incubate at room temperature for 15 minutes.
4. Add 1.4 μL of β-mercaptoethanol to quench residual EDC activity.
5. Remove excess EDC, NHS, and β-mercaptoethanol using molecular sieve chromatography.

Conjugation with Hapten

1. Add an equimolar amount of hapten (dissolved in PBS) to the activated SHC solution and incubate at room temperature for 2 hours.
2. Add hydroxylamine to a final concentration of 10 mM to terminate the reaction.
3. Remove excess hydroxylamine using molecular sieve chromatography.
4. Add 1.4 μL of β-mercaptoethanol to deactivate EDC in the reaction mixture.
5. Remove excess EDC, NHS, and β-mercaptoethanol using molecular sieve chromatography.

Note

This product is accurately quantified. Dissolve and use directly in the original vial at the desired concentration. Do not divide or transfer the product, as this may result in material loss due to product properties or static electricity.