YamayBio

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

QUICK START GUIDE

Research use only.
Not for diagnostic procedures.

Contents and storage

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

SH2170-10mg

SH2170-100mg

SH2170-100mg×6

Storage: Store at -20°C. Product is shipped at ambient temperature.

Introduction

YamayBio Mariculture SHC is a purified shrimp (*Penaeus vannamei*) hemocyanin. It can serve 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, imparting high immunogenicity to them. Testing has shown that SHC exhibits comparable immunogenicity to KLH, while its solubility (especially after conjugation with haptens) is significantly higher than that of KLH, providing greater flexibility in immunogen preparation protocols.

Molecular Weight: 70KD and 73KD

Appearance: Dark blue powder

Purity (MPLC-SEC): ≥98%

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

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

Endotoxin Level:≤11.7 USP-EU/mg

Product Form: The hemocyanin is provided in lyophilized form. It can be reconstituted with ultrapure water.

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 prepare a 10 mg/mL SHC solution.
2. Add 2 mL of freshly prepared SMCC solution (5 mg/mL in ultrapure water) to the 2 mL 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 hapten with thiol groups 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 react at room temperature for 2 hours. Remove excess SMCC using molecular sieve chromatography.
3. Remove EDTA using molecular sieve chromatography.

Note that 0.1 mM TCEP is not necessary, which is used to treat the hapten for reducing the disulfide bond to the thiol group.

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 prepare a 1 mg/mL SHC solution.
3. Add 0.4 mg of EDC and 0.6 mg of NHS to the 1 mL prepared SHC solution, mix well, and react at room temperature for 15 minutes.
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.

Conjugation with Hapten

1. Add an equimolar amount of hapten (dissolved in PBS) to the activated SHC solution and react at room temperature for 2 hours.
2. Add hydroxylamine (final concentration of 10 mM) to the reaction mixture 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. Please dissolve and use directly in the original vial according to the desired concentration. Do not divide or transfer this product, as it may cause significant loss due to product characteristics or static electricity.