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Product Information

Maleimide Activated KLH

Catalog Number **K0383**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Synonym: Maleimide activated keyhole limpet hemocyanin

Product Description
Low molecular mass molecules, such as peptides, are often not sufficiently immunogenic to elicit an immune response alone. Synthetic peptides (1–3 kDa) are widely used to generate antibodies and can be made immunogenic by conjugation to a suitable carrier. A wide range of proteins, synthetic polymeric carriers, and conjugation methods are available to prepare immunogens from non-immunogenic small haptens.

Hemocyanins are multimeric, high molecular mass, oxygen transport metalloproteins. KLH, from the hemolymph of the marine mollusc *Megathura crenulata*, is expressed as two subunit isoforms (KLH1 and KLH2) of 350–400 kDa. The KLH monomers each contain 7 or 8 functional unit domains, each functional unit containing an oxygen binding site carrying two copper atoms. Both KLH isoforms can assemble into multimeric forms containing native decamers of $4–8 \times 10^6$ Da. Higher multimeric forms have also been described. KLH is often used as a carrier protein due to its highly immunogenic properties and the large number of lysine residues available for modification.

Maleimide activated KLH has reactive maleimide groups on its surface, available for conjugation with a cysteine-containing peptide or a thiol-containing hapten. The reaction of the maleimide group and the thiol group proceeds rapidly and selectively under mild coupling conditions (pH 6.5–7.5) to yield a stable, covalently linked peptide-protein conjugate that may be used in immunization protocols or in antibody screening methods.

Maleimide Activated KLH is provided as a lyophilized reagent, eliminating the need for time-consuming activation and purification steps of the carrier protein. Maleimide Activated KLH contains 160–320 maleimide groups for each KLH molecule. The conjugation reaction with a cysteine containing peptide is performed at pH 6.6–7.0.

The extent of conjugation can then be determined by a colorimetric assay with Ellman's reagent to detect unreacted thiol groups in the cysteine-containing peptide.

The product is lyophilized from 10 mM sodium phosphate buffer, pH 6.6, with 115 mM NaCl, 1 mM EDTA, and 40 mM sucrose as stabilizer.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The lyophilized product should be stored at 2–8 °C.

Do not store the reconstituted Maleimide Activated KLH solution as the reactive maleimide group is not stable in solution. Use immediately upon reconstitution. Mix the protein solution gently to prevent its denaturation and aggregation.

Procedure
Conjugation to Maleimide Activated KLH
The following procedure describes the conjugation of a synthetic peptide containing cysteine to maleimide activated KLH. A molar ratio in the reaction mixture of ~3,000:1 for peptide (MW 1,500) to maleimide activated KLH is recommended. This ratio corresponds to ~10 peptide-Cys moieties available to react with each maleimide activated site. This usually results in complete conjugation and provides an immunogen with a high hapten density on the carrier surface. This procedure can be modified for a peptide of different molecular mass, for a different amount of peptide, or if a lower molar ratio is desired in the reaction.
Note: See Troubleshooting Guide for suggestions on handling peptide solutions.

1. Slowly open the vial of Maleimide Activated KLH to release the vacuum.
2. Reconstitute the contents of the vial with 1 mL of water to obtain a 5 mg/mL solution of maleimide activated KLH in 20 mM sodium phosphate buffer with 230 mM NaCl, 2 mM EDTA, and 80 mM sucrose, pH 6.6. Do not mix by vortex. Use immediately.
3. Dissolve 4 mg of cysteine-containing peptide (~1,500 Da) in 0.5 mL of conjugation buffer containing 20 mM sodium phosphate buffer with 100 mM EDTA and 80 mM sucrose, pH 6.6, or alternatively in water (see Troubleshooting Guide, Note 4). If required retain 50 µL of the peptide solution for determination of coupling efficiency.
4. Immediately mix peptide solution with the Maleimide Activated KLH solution in a reaction vial equipped with a stirring bar. De-gas sample for 1–2 minutes while stirring under a gentle nitrogen stream.
5. Cap reaction vial and continue stirring for 2 hours at room temperature or overnight at 2–8 °C. If required, retain 100 µL of the peptide solution for determination of coupling efficiency.
6. Optional steps – The peptide-carrier conjugate can be isolated from the unconjugated peptide and the extent of conjugation can be determined.
   a. Isolation of the conjugate is done by a single gel filtration step on a Sephadex® G-25M column or by dialysis against an appropriate buffer (e.g., PBS, pH 7.4).
   b. The extent of conjugation can be determined using Ellman's reagent.

   Note: Detailed procedures for the isolation of the conjugate and determination of the extent of conjugation (Ellman's test) can be found in the Technical Bulletin for **Maleimide Activated BSA, KLH Conjugation Kit, Catalog Number MBK1**.

Troubleshooting Guide

Notes on handling peptides and peptide solutions.

1. Synthetic peptides used in the conjugation reaction should be of the highest possible purity (HPLC purified, >90%) to avoid unwanted side reactions (e.g., formation of color, turbidity, or aggregates).
2. It is suggested to determine the extent of peptide solubility and stability in aqueous solutions on a small scale (e.g., 1–2 mg of peptide) before proceeding to the conjugation reaction.
3. Some cysteine peptides do not readily dissolve in aqueous solutions and/or tend to rapidly oxidize the cysteine residues to form disulfide containing dimers, that may precipitate out from the solution.
4. Peptide solutions should not be prepared in buffers containing thiols, sodium azide, or amines such as Tris or glycine, since they compete with the conjugation reaction.
5. It is not recommended to dissolve peptides directly in PBS buffers. Dissolve in water or in conjugation buffer.
6. Slightly insoluble peptides can be initially dissolved in an organic solvent. Highly purified, amine-free, water miscible solvents such as *N*-methyl-pyrrolidone (NMP) or *N*,*N*-dimethylformamide (DMF) can be used. The organic solvent should not exceed 10% of the conjugation reaction volume.
7. Do not dissolve cysteine-containing peptides in dimethyl sulfoxide (DMSO). This may lead to a rapid oxidation of the sulfhydryl groups.
8. Avoid prolonged storage of peptides in solution due to their tendency to form aggregates or to oxidize.
9. Oxidized cysteine-peptides of sulfhydryl haptens must be reduced with 2-mercaptoethanol or dithiothreitol (DTT), and purified by HPLC prior to use for conjugation.

References

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2. Green, N. et al., Cell, **28**, 477-487 (1982).
3. Moroder, L. et al., Biopolymers, **22**, 481-486 (1983).
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5. Van Regenmortel, M. et al., in Synthetic Polypeptides as Antigens, Burdon, R., and Knippenberg, P., eds., (1988).

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