

Imject[®] Maleimide Activated mcKLH

77605 77606 77610 77663

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Number	Description
77605	Imject Maleimide Activated mcKLH , 10mg, store at 4°C
77606	Imject Maleimide Activated mcKLH , 2mg, store at 4°C
77610	Imject Maleimide Activated mcKLH , 10 × 10mg, store at 4°C
77663	Imject Maleimide PEGylated mcKLH , 10mg, store at -20°C
Supplied: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA and 50mM sucrose; pH 7.2	
Activation level: ≥ 400 moles of maleimide/mole of mcKLH	

Storage: Upon receipt store product as indicated above. Product is shipped with an ice pack.

Introduction

The Thermo Scientific Imject Maleimide Activated mcKLH (see Appendix A for more information on carrier proteins) is for conjugating a sulfhydryl-containing hapten to elicit an immune response and antibody production against the hapten. The mcKLH is maleimide-activated using a heterobifunctional crosslinker that contains an *N*-hydroxysuccinimide (NHS) ester and a maleimide group. The PEGylated mcKLH is activated with NHS-PEG₂-Maleimide, creating a more soluble maleimide-activated mcKLH. Maleimide groups react with free sulfhydryls, forming stable thioether bonds.

Keyhole limpet hemocyanin (KLH), a copper-containing protein, belongs to a group of non-heme proteins that are present in arthropods and molluscs. Mariculture KLH (mcKLH) is harvested from the hemolymph of giant keyhole limpets (*Megathura crenulata*) that are maintained in environmentally optimized marine tanks. These limpets are not sacrificed during hemolymph collection and can supply samples over an extended period.

Important Product Information

- Maleimides react with sulfhydryls at pH 6.5-7.5 to form stable thioether bonds. At pH values > 7.5, reactivity toward primary amines and hydrolysis of the maleimide group can occur.
- Haptens must have free sulfhydryl (–SH) group(s) available. Cysteine-containing peptides often oxidize in solution and form disulfide bonds, which cannot react with maleimides. Disulfide bonds can be reduced to produce free sulfhydryls. After reduction, most reducing reagents must be removed before conjugation. The Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enables peptide or protein reduction while recovering the sample in the absence of reducing agents.
- Sulfhydryls can be added to molecules using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101). Both of these reagents modify primary amines.

Procedure for Hapten-Carrier Conjugation with Maleimide Activated mcKLH

The protocol is designed to yield effective immunogens for the widest variety of haptens but is not necessarily optimal for a specific hapten. Differences in the sizes and structure of haptens will affect conjugation efficiencies. Using a molar excess of hapten (peptide) over maleimide groups on the mcKLH ensures complete and efficient conjugation. Generally, reacting equal mass amounts of hapten and carrier protein will achieve sufficient molar excess. If a molar excess of hapten is not possible, add a sulfhydryl-containing compound, such as cysteine, after conjugation to quench any remaining active maleimide groups.

A. Additional Materials Required

- Conjugation Buffer: Phosphate-buffered saline (PBS; 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2; Product No. 28372), or other buffer at pH 6.5-7.5
- Desalting column (Product No. 43240) or dialysis cassettes (Product No. 66382)
- Inject Purification Buffer Salts (Product No. 77159), contains 0.083M sodium phosphate, 0.9M sodium chloride and sorbitol (see note below)

Note: If the conjugate is to be used for injection within one week, use PBS for desalting. If the conjugate will be frozen, use the Purification Buffer Salts for desalting, which will preserve the product during freeze-thaw cycles.

B. Conjugation Procedure

1. Add water to the Maleimide Activated mCKLH to make a 10mg/mL solution. The PEGylated mCKLH can be reconstituted up to 20mg/mL, if needed.

Note: mCKLH forms a suspension that typically appears translucent to whitish blue. Do not vortex or heat the suspension, which will cause the mCKLH to precipitate.

2. Dissolve the sulfhydryl-containing hapten in a volume of Conjugation Buffer equal to 1.0-2.5 times the volume of reconstituted mCKLH. For example dissolve 2mg of peptide in 200-500µL of buffer for adding to 2mg of activated mCKLH reconstituted in 200µL water. Alternatively, if the peptide is freely soluble add it as a solid to the mCKLH suspension.

Note: For haptens with limited solubility, DMSO may be used for solubilization. Use ≤ 30% DMSO in the final conjugation solution or the carrier protein may irreversibly denature.

3. Immediately mix the peptide and activated mCKLH and react for 2 hours at room temperature.
4. Purify conjugate by gel filtration or dialysis to remove EDTA, which is an anti-coagulant and should not be injected into laboratory animals.

C. Conjugate Purification by Desalting

- Use either desalting or dialysis to remove EDTA. If DMSO was used in the conjugation, add DMSO to the Purification Buffer Salts for desalting to prevent precipitation in the column; dialysis is not compatible with DMSO. Desalting or dialysis will not separate non-conjugated mCKLH; however, a large excess of hapten is used in this protocol, making it unlikely that non-conjugated carrier exists in significant quantity.
 - If the conjugate is to be used for injection within one week, PBS may be used for purification. If the conjugate will be frozen, use Purification Buffer Salts for purification, which will preserve the product during freeze-thaw cycles.
 - If a precipitate formed during conjugation, centrifuge the precipitated material, collect the supernatant and save the precipitate. Purify only the supernatant. Combine the purified conjugate to the precipitate.
1. For desalting, dissolve contents of one bottle of Purification Buffer Salts by adding 60mL degassed, ultrapure water to the bottle. Store excess buffer at 4°C.
 2. Sequentially remove the top and bottom caps from a desalting column and allow the storage solution to drain. Use one desalting column for each 0.5mL of sample.
 3. Wash column with 3-5 column volumes (i.e., 15-25mL) of Purification Buffer.
 4. Apply 0.5mL of the hapten-carrier mixture directly to the center of the column disc. Add 8-10 aliquots of 0.5mL of Purification Buffer and collect each fraction in a separate tube.
 5. Measure absorbance at 280nm to locate fractions containing the conjugate. The hapten-carrier conjugate will be in the first absorbance peak detected. Pool all fractions that contain acceptable levels of conjugate.
 6. After the conjugate-containing fractions have emerged, the non-conjugated hapten can be recovered by continuing to add buffer to the column and collecting additional fractions.
 7. If the immunogen is to be stored for more than a few days, sterile filter the conjugate fractions and store in a sterile container at 4°C or -20°C. See Appendix B for immunization suggestions.

Note: To purify the antibodies specific to the peptide, prepare an affinity column by immobilizing the peptide through the same functional group used to prepare the immunogen (see Appendix C).

Appendix

A. Carrier Proteins

Small molecules (haptens) such as peptides, are able to interact with products of an immune response, but cannot stimulate a response alone. Haptens can be made fully immunogenic by coupling them to a suitable carrier molecule. Some of the more common carrier proteins include keyhole limpet hemocyanin (KLH; 4.5×10^5 - 1.3×10^7 Da), bovine serum albumin (BSA; 67,000 Da) and ovalbumin (OVA; 45,000 Da). KLH is the most widely used carrier because of its large molecular mass, strong immunogenicity and many available lysines for conjugation.

KLH exists as five different aggregate states in Tris•HCl buffer, pH 7.4 and reversibly dissociates to subunits with moderate changes in pH; at pH 8.9 it will completely and irreversibly dissociate to subunits. Each subunit contains oxygen-binding sites; one molecule of oxygen can be bound for every two copper atoms in KLH. The oxygen-containing protein is blue, while the oxygen-lacking form is colorless. Removal of oxygen will dissociate subunits into lower aggregation states. Increased antibody binding occurs when KLH is dissociated to its subunits because of improved availability of antigenic sites.

Individual mcKLH subunits are ~450,000 Da and associate to form didecamers with an approximate molecular weight of 8×10^6 . We formulate the mcKLH to dissolve quickly without the formation of precipitates that are often present in other commercial KLH products. Soluble mcKLH appears opalescent blue when dissolved.

B. Immunization for Mice and Rabbits

After preparing the immunogen with an adjuvant (see Related Products section), the following protocol may be used for immunization.¹ This schedule is a general protocol for immunization and may be customized as needed.

CAUTION: Only qualified personnel should perform these procedures. Individuals unfamiliar with these techniques should consult an experienced investigator for training before attempting to immunize and bleed animals.

Day 0: Collect non-immune serum to be used as a blank for ELISAs and store frozen.

For mice, make initial injection of 50-100µg of immunogen per mouse in one to two injections. Intraperitoneal and subcutaneous injection sites are the most common for mice.

For rabbits, make an initial injection of 100µg of immunogen into 6-10 subcutaneous sites on the animal's back.

Day 14: Boost with same sample size as the initial injection. If Freund's Complete Adjuvant was used for the initial injection, use Freund's Incomplete Adjuvant for boosts to avoid animal stress.

Day 21: Test bleed and screen for antibody response by ELISA. For mice, 200-400µL samples from the tail vein or the retro orbital plexis are appropriate. For rabbits, 5-10mL samples from the ear vein may be obtained.

Day 28: Boost again if necessary. Continue to periodically bleed and boost until a satisfactory immune response occurs.

C. Purification of Peptide-specific Antibodies

Using a carrier protein for antibody production also results in the production of antibodies against the carrier protein. To purify the antibodies specific to the peptide, immobilize the peptide through the same functional group used to prepare the immunogen. The Thermo Scientific SulfoLink Kit for Peptides (Product No. 44999) contains an activated resin that will couple peptides via sulfhydryl groups. The peptide affinity column can then be used to specifically bind anti-peptide antibodies from serum, allowing antibodies against the carrier protein to flow through the column. Elution of peptide-specific antibodies can then be accomplished with an elution buffer, such as IgG Elution Buffer (Product No. 21009) or Gentle Elution Buffer (Product No. 21027).

D. Information available from the internet

Visit our web site for additional information relating to this product including the following items:

- Tech Tip #7: Remove air bubbles from columns
- Tech Tip #29: Degas solutions for use in affinity columns
- Tech Tip #26: Prepare a measurable and reversible sulfhydryl-reactive affinity column
- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

77140	Imject Freund's Complete Adjuvant , 5 × 10mL
77145	Imject Freund's Incomplete Adjuvant , 5 × 10mL
77161	Imject Alum , 50mL
66382	Slide-A-Lyzer Dialysis Cassette Kit , 10 dialysis cassettes, each appropriate for 0.5-3.0mL samples
45212	Melon™ Gel IgG Purification Kit , sufficient reagents to purify IgG from up to 50mL of serum
45206	Melon Gel IgG Spin Purification Kit , sufficient reagents to purify up to 3mL of serum
44999	SulfoLink Kit for Peptides , for immobilizing sulfhydryl-containing peptides, contains sufficient reagents for preparing five affinity columns

Cited Reference

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General References

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