

Maleimide Activated KLH

Catalog #	Source	Reactivity	Applications
CRG1128			
Description	Maleimide Activated Carrier Proteins are activated using the crosslinker Sulfo-SMCC, which contains a maleimide group		
Directions for Use	<p>General Procedure for Peptide-Carrier Conjugation</p> <ol style="list-style-type: none"> 1. Reconstitute maleimide-activated carrier protein. Reconstitute with 1mL of ultrapure water for 10 mg Maleimide Activated KLH to yield a solution containing 10mg/mL. Dilute with a buffer containing 0.1M sodium phosphate, 50 mM EDTA at pH 6.8 for concentrations < 10mg/mL. 2. Use up to 2mg peptide/2mg of activated carrier. Dissolve up to 2mg of sulfhydryl-containing peptide per 200-500µL of physiological pH phosphate buffer. Alternatively, add the peptide as a solid to the activated carrier solution if it is soluble. 3. Immediately mix the peptide and activated carrier solutions and allow them to react for 2 hours at room temperature. 4. To remove EDTA from the activated protein, purify the conjugate by gel filtration or dialysis. EDTA is an anti-coagulant and should not be injected into laboratory animals. Note: Dialysis minimizes sample loss and clogging of the desalting column when conjugating hydrophobic peptides. Desalting or dialysis will not separate KLH from the conjugated product; however, a large excess of peptide is used in this protocol, making it unlikely that non-conjugated carrier exists in significant quantity. 5. If the conjugate is to be stored for several days, sterile filter the conjugate fractions and store them in a sterile container at 4°C or frozen at -20°C. For extended storage, lyophilize the conjugate and store at 4°C. 		
Precautions for Use	1. 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		

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group can occur; however, the maleimide group of Sulfo-SMCC is unusually stable up to pH 7.5.

2. Molecules for conjugation must have free sulfhydryl (–SH) group(s) available. Cysteine-containing molecules 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.
3. PBS may be used for conjugate purification. If the conjugate will be frozen, use the Purification Buffer Salts, which will preserve the product during freeze-thaw cycles.
4. Desalting or dialysis will not separate non-conjugated protein; however, a large excess of hapten is used in this protocol, making it unlikely that non-conjugated carrier exists in significant quantity.
5. 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.
6. If a precipitate has formed during conjugation, centrifuge the material, collect the supernatant and save the precipitate. Use only the supernatant for purification. Combine the purified conjugate to the precipitate.

Storage

Store at -20°C for one year.

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