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TECHNICAL INFORMATION

Catalog Number: 151233
Hemocyanin, keyhole limpet

CAS # : 9013-72-3

Molecular Weight: 3,000,000 - 7,500,000

Synonym: KLH; Haemocyanin

Physical Description: White to gray lyophilized powder

Source: Keyhole Limpet (*Megathura crenulata*)

Physical Appearance: Pale blue-gray lyophilized powder

Protein Concentration: ~ 85% (wt/wt)

Copper to Protein Ratio: ~ 2.5 X 10⁻³

Buffer Concentration: Lyophilized at ~ 30 mg/ml (protein) in 5 mM BES buffer, pH 6.5 and 10 mM MgSO₄.

Reconstitution: Dissolve at ~30 mg/ml (protein) in distilled water or dissolve 5-10 mg/ml in PBS or other physiological by gentle addition of the buffer (i.e. slowly added down the side of the tube.)

DO NOT vortex or agitate vigorously to dissolve product. (KLH is an extremely large protein complex and has a tendency to aggregate and precipitate from solutions). For further dilutions, use an appropriate buffer and maintain the magnesium concentration at ≥ 10 mM. Solutions should be stored at +4°C.

Description: Hemocyanins are copper-containing oxygen transport proteins found in the hemolymph of many invertebrates. It is dissolved in the hemolymph; not found in blood cells.¹ They are divided into 2 main groups, arthropodan (such as horseshoe crab) and molluscan (such as keyhole limpet). These have structurally similar oxygen-binding sites, which are similar to the oxygen-binding sites of tyrosinases⁴, but their quaternary structures are arranged differently. The arthropodan proteins exist as hexamers comprising 3 heterogeneous subunits (a, b and c) and possess 1 oxygen-binding site per subunit. The molluscan protein exists as cylindrical oligomers of 10 to 20 subunits and possess 7 or 8 oxygen-binding sites per subunit.⁸ Although the proteins have similar amino acid compositions, the only real similarity in their primary sequences is in the region corresponding to the second copper-binding domain, which also shows similarity to the copper-binding domain of tyrosinases.⁴ The molluscan hemocyanin consists of two immunologically and physiologically distinct isoforms, KLH1 and KLH2.⁵ The oxygen is bound between two copper ions, forming a Cu(II)-O₂²⁻-Cu(II) complex.² The deoxygenated Cu(I) is colorless and the oxygenated Cu(II) form is blue. The oxygenated active sites create two spectroscopic signals indicating the oxygen load of the hemocyanins: first, an absorption band at 340 nm which is due to a ligand-to-metal charge transfer complex, and second, a strong quenching of the intrinsic tryptophan fluorescence, the cause of which has not been definitively identified.²

KLH has many available primary amines to facilitate protein conjugation. KLH is used to immunize animals to elicit antibodies to small molecules (haptens) by covalent conjugation. Haptens such as peptides can be coupled to KLH using a wide range of cross-linking reagents and conjugation methods.^{10,11}

Good coupling methods are glutaraldehyde (amino to amino), m-Maleimidobenzoic acid-N-hydroxysuccinimide (amino to sulfhydryl), bisdiazobenzidine (couples tyr to tyr), EDAC (carbodiimide; couples amino to carboxyl).

Note: when coupling KLH to a peptide an extra tyr to the N- or C- terminus of the synthetic peptide, for bisdiazobenzidine coupling, has been used. The extra residue can be attached to the end of least interest since it will be attached to the carrier, and hence you will tend to get antibody to the end of most interest (away from the carrier).

Availability:

Catalog Number	Description	Size
151233	Hemocyanin, Keyhole limpet	50 mg 100 mg 250 mg

Also Available:

Catalog Number	Description	Size
618101	Rabbit anti-keyhole limpet hemocyanin	1 ml
55966	Rabbit anti-keyhole limpet hemocyanin	2 ml

References:

- Merck Index, **13th Ed.**, No 4665.
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- Gaykema, W.P.J., et al., "Angstrom structure of the copper-containing oxygen-carrying protein Panulirus interruptus haemocyanin." *Nature*, **v. 309**, 23-29 (1988).
- Gebauer, W., et al., "Quaternary structure, subunits and domain patterns of two discrete forms of keyhole limpet hemocyanin: KLH 1 and KLH2." *Zoology*, **v. 98(1)**, 51-68 (1994).
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