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 \times 10^{-3}
**Buffer Concentration**: Lyophilized at ~30 mg/ml (protein) in 5 mM BES buffer, pH 6.5 and 10 mM MgSO_4.

**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. 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 tyrosinases, 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. 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. The molluscan hemocyanin consists of two immunologically and physiologically distinct isoforms, KLH1 and KLH2. The oxygen is bound between two copper ions, forming a Cu(II)-O_2^-Cu(II) complex. 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.

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.

**Availability**:

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<th>Description</th>
<th>Size</th>
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<td>Hemocyanin, Keyhole limpet</td>
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**Also Available**:

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<td>55966</td>
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**References**:

– *Merck Index, 13th Ed.*, No 4665.