3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

HEMOGLOBIN

Since native hemoglobin is readily oxidized in air, these preparations may be predominantly methemoglobin.

PRODUCT NUMBER	NAME	DESCRIPTION
H2500	Hemoglobin, Bovine	Lyophilized powder
H3760	Hemoglobin, Bovine	Dried erythrocytes Methemoglobin and oxyhemoglobin content is not determined
H2625	Hemoglobin, Bovine	Substrate powder Prepared from washed, lysed and dialyzed erythrocytes. Suitable for use as a protease substrate.
H3636	Hemoglobin, Cat	Lyophilized powder
H7130	Hemoglobin, Dog	Lyophilized powder
H1887	Hemoglobin, Garter Snake	Lyophilized powder
H2260	Hemoglobin, Goat	Lyophilized powder
H4632	Hemoglobin, Horse	Lyophilized powder
H7379	Hemoglobin, Human	Lyophilized powder Tested negative for HIV and Hepatitis B antigen.
H5633	Hemoglobin, Mouse	Lyophilized powder
H4131	Hemoglobin, Porcine	Lyophilized powder
H0256	Hemoglobin, Pigeon	Lyophilized powder
H7255	Hemoglobin, Rabbit	Lyophilized powder
H3883	Hemoglobin, Rat	Lyophilized powder
H2750	Hemoglobin, Sheep	Lyophilized powder
H0142	Hemoglobin, Turkey	Lyophilized powder

HEMOGLOBIN

CAS NUMBER: 9008-02-0

SYNONYMS: Hb; Ferrohemoglobin

PHYSICAL PROPERTIES:

Appearance: Red-brown powder
pI: 6.8[4]
UV Data: Extinction coefficients of hemoglobin from different species vary due to differences in amino acid composition and sequence of the globin chains.[1] Spectral assays of oxyHb, deoxyHb and ferriHb have been reported in the literature.[6,7] Extinction coefficients are strongly pH-dependent.[6]
Molecular weight: Mammalian hemoglobins have molecular weights of ~64,500.[1]

SOLUBILITY / SOLUTION STABILITY:

Hemoglobin is soluble in water (1 part in 7 of water), and slowly soluble in glycerol.[1] Sigma tests the solubility in water or in 100 mM phosphate buffer at 20 mg/mL and obtains dark red-brown solutions. No solution stability data are currently available.

STRUCTURE:

Hemoglobin is a tetramer composed of 2 pairs of polypeptide chains called globins, and 4 heme groups. Each polypeptide chain is bound to one heme. Iron is coordinated to 4 pyrrole nitrogens of protoporphyrin IX and to an imidazole nitrogen of a histidine residue from the globin side of the porphyrin. The sixth coordination position is available for binding with other small molecules such as O_2, CO or CO_2; the hemoglobin distorts in the process. Adult human hemoglobin consists of 96.5-98.5% HbA_1 ($a_2ß_2$ dimer) and 1.5-3.5% HbA_2 (a_2d_2 dimer), where a, ß and d refer to subunits with different amino acid sequences.[1,2,3]

NOMENCLATURE OF HEMOGLOBIN FORMS:

1. Ferrihemoglobin, also called methemoglobin, refers to hemoglobin which contains iron in the +3 oxidation state (Fe^{3+}).

2. Ferrohemoglobin, also called reduced hemoglobin, refers to hemoglobin which contains iron in the +2 (or reduced) oxidation state (Fe^{2+}). Hemoglobin must be in the reduced form to bind oxygen or other small molecules:

   a. Oxyhemoglobin (HbO_2) contains bound oxygen;

   b. Deoxyhemoglobin (Hb) does not contain bound oxygen;

   c. Carboxyhemoglobin (HbCO) contains carbon monoxide, which has displaced oxygen. The affinity of Hb for CO, a poisonous gas, is 325 times greater that its affinity for O_2.[2]

HEMOGLOBIN

METHOD OF PREPARATION:

Hemoglobin is usually prepared by separating red blood corpuscles from the lighter plasma components by centrifugation. The plasma is siphoned off and ether is added to the corpuscle paste, causing the cells to burst. Another centrifugation removes the ruptured cell envelopes, and leaves a clear red solution of hemoglobin.[1] References for methods of preparation of oxyhemoglobin (HbO_2) from horse, dog and human erythrocytes have been published.[1]

PRODUCT DESCRIPTION:

Hemoglobin is the major component of red blood cells, and is responsible for their red color. Its normal concentration in erythrocytes is 34%. Hemoglobin is the most important respiratory protein of vertebrates by virtue of its ability to transport oxygen from the lungs to body tissues, and to facilitate the return transport of carbon dioxide. The hemoglobin of all human races and chimpanzees are identical. Anomalous globins in which various amino acids have been substituted with others, or in which certain amino acids are missing entirely from the normal sequence, comprise 153 abnormal hemoglobin species. Some of these are responsible for diseases, the most common of which is sickle cell anemia - a condition which affects about 10% of Americans of African ancestry. In sickle cell hemoglobin (HbS), a valine residue has replaced the glutamic acid residue at position 6 in the ß-chain of normal Hb; the a-chain is normal. About 0.5% of all humans carry a mutant hemoglobin.[2,3]

PREPARATION OF REDUCED HEMOGLOBIN (HbO_2) FROM OXIDIZED HEMOGLOBIN:[5]

1. Equilibrate a 25 X 2.5 cm column of Sephadex G-25 with 20 mM phosphate buffer, pH 7.0, containing 10^{-3} M EDTA.
2. Apply to the column 2 mL of the same buffer to which 200 mg of sodium dithionite have been added, and help it drain into the gel by adding 1 mL of the phosphate buffer.
3. Apply to the column about 10 mL of sample containing oxidized hemoglobin and elute with the phosphate buffer.
4. Saturate the reduced hemoglobin eluent with oxygen gas.
5. Dialyze the oxygenated eluent against oxygen-saturated phosphate buffer to eliminate excess dithionite and achieve complete conversion to oxyhemoglobin.

REFERENCES:

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2. T. Scott and M. Eagleson, *Concise Encyclopedia: Biochemistry*, 2nd Ed., pp. 255-259, Walter de Gruyter Press, New York (1988).
3. A. L. Lehninger, *Biochemistry*, 2nd Ed., p. 111, Worth Publishers, Inc., New York (1975).
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5. H.B.F. Dixon and R. McIntosh, *Nature*, 213, 399 (Jan. 28, 1967).
6. Benesh, R.E., R. Benesh and S. Yung, *Anal. Biochem.*, 55, 245 (1973).
7. D.L. Drabkin, et al., *J. Biol. Chem.*, 185, 231 (1950).