

**Quantitative Determination of Hemoglobin in Whole Blood  
Only for *In Vitro* Diagnostic use**

**ORDER INFORMATION**

REF	Cont.
HB 500	1 X 500 ml
HB 1000	1 X 1000 ml

**CLINICAL SIGNIFICANCE**

The hemoglobin is a protein that contains iron and that the red color to the blood. The hemoglobin is in red globules and it is the one in charge of oxygen transport by the blood from the lungs to weaves. When the level of hemoglobin appears underneath the normal levels is describing an anemia that can be of different origins: primary anemia, cancer, pregnancy, renal diseases, and hemorrhages. If the hemoglobin levels appear high it can be due to: cardiopathies, dehydration and stays in places of much altitude. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

**PRINCIPLE**

In the cyanomethemoglobin method, erythrocytes are lysed by a stromatolytic reagent in the presence of a surfactant and release their hemoglobin in to the solution. Hemoglobin is oxidised to methemoglobin by ferricyanide, and the methemoglobin is converted into the stable cyanmethemoglobin by addition of KCN. The absorbance of cyanmethemoglobin is measured at 540 nm and the color intensity is directly proportional to hemoglobin concentration.

**REAGENT COMPOSITION**

Reagent I : Cyanomethemoglobin reagent

**SAFETY PRECAUTIONS AND WARNINGS**

Potassium cyanide: Harmful (Xn): R26/27/28: Very toxic by inhalation, in contact with skin and if swallowed. R32: Contact with acids liberates very toxic gas.  
Environmentally dangerous (N): R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.  
S7: Keep container tightly close. S28: After contact with skin, wash immediately with plenty of water.S29: Do not empty into drains. S45: In case of accident or if you feel unwell, seek medical advice immediately. S60: This material and its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/safety data sheets.  
Cyanide (poison): The amount of cyanide in the Reagent Concentrate (50x) is appreciably less than the minimum lethal dose for an adult. Gaseous hydrogen cyanide will be released on contact with acids.

**SAMPLE COLLECTION AND PRESERVATION**

Collect whole blood with EDTA using aseptic technique. Whole blood collected with EDTA is stable for one week at 2 - 8°C.

**REAGENT PREPARATION**

The reagents are ready to use.

**REAGENT STORAGE AND STABILITY**

The reagent stability is till expiry, if stored at R.T. (away from sunlight).

AUTOMATED PARAMETERS	
Wavelength	540 nm
Reaction Type	End point
Cuvette	1 cm light path
Reaction Temperature	R.T.
Measurement	Against reagent blank
Sample Volume	20 µl
Reagent Volume	5000 µl
Incubation	5 minutes
Factor	36.7
Low Normal	10.0 g/dl
High Normal	18.0 g/dl
Linearity	20.0 g/dl

**MANUAL ASSAY PROCEDURE**

**PIPETTE INTO TEST TUBES**

	BLANK	SAMPLE
Sample	-	20 µl
Standard	-	-
Reagent	5000 µl	5000 µl

Mix, Incubate at R.T. for 5 min. Measure final absorbance of the sample (Ac) against the reagent blank. at 540 nm ( 520 - 550 nm)

**CALCULATION**

$Ac \times 36.7 = \text{g/dl HEMOGLOBIN}$
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**LINEARITY**

This method is linear to a concentration of 20 g/dl. if the hemoglobin values are > than 20 g/dl, dilute 1:1 with deionised water. Multiply result by 2.

**INTERFERING SUBSTANCES**

Falsely elevated values are found in lipaemic samples, abnormal plasma proteins etc. Numerous drugs exert an in vivo effect to decrease blood hemoglobin.

**QUALITY CONTROL**

The integrity of the reaction should be monitored by use of control material (normal/abnormal) with known hemoglobin concentrations.

**REFERENCE VALUES**

Adult Males	13.0 - 18.0 g/dl
Adult Females	11.0 - 16.0 g/dl
Children	10.0 - 14.0 g/dl
Newborns	14.0 - 23.0 g/dl

**BIBLIOGRAPHY**

Eilers R.J., Am. J. Clin. Path ., 47 :212 (1967).  
Tietz N.W., Fundamentals of Clinical Chemistry.  
2nd ed. W.B. saunders Co., Philadelphia p 411 (1976)