

HAEMOGLOBIN REAGENT KIT (Cyanomethanoglobin method)

For photometric determination of Haemoglobin in whole blood

Only for In Vitro diagnostic use

Ref no

Hb500

Hb1000

Summary

Haemoglobin is the major source of oxygen for various tissue cells and its deficiency leads to the destruction of tissue cells. Increased levels are found in polycythaemia vera, congenital cyanotic heart disease, heat stroke and dehydration. Decreased levels are found in all varieties of anemias, resulting from deficiency of iron or folic acid, red blood hemolysis, defective globin synthesis and structural abnormalities.

Principle

Potassium ferricyanide converts haemoglobin to methaemoglobin. The methaemoglobin further reacts with potassium cyanide to produce a stable cyanmethaemoglobin complex. Intensity of the complex formed is directly proportional to the amount of haemoglobin present in the sample.

Kit Contents

Kit size	500ml	1000ml
Ref no.	Hb500	Hb1000
Haemoglobin	1	1
IFU	1	1

Material required but not provided

1. Accurate pipetting devices
2. Photometer capable of reading at 546 nm
3. Test Tubes, yellow tips, blue tips

Storage & Stability of the Reagents

1. The reagent is stable till the date of expiry, when stored at 15^o-20^oC, protect from light & contamination is avoided.
2. Do not freeze the reagents.
3. Avoid Contamination of Ready-To-Use Reagents.

4. Always use fresh pipette tips. Keep always the caps tightly closed.
5. Ensure the reagents & specimens are brought to Room Temperature.
6. Ensure the reagents shelf life is valid.
7. Do not use haemolysed & lipemic serum.

Reagent preparation

The reagents are ready to use

Reagent Composition

Reagent	KH ₂ PO ₄	0.1g/l
	Potassium cyanide	0.044g/l
	Triton x-100	500ml/l

Specimen

Whole blood. Preferably fresh and collected in EDTA.

Specimen collection

- Whole blood from venipuncture may be used if mixed immediately with suitable anticoagulants such as heparin, oxalate, Citrate or EDTA.
- Capillary blood taken from finger puncture must be pipetted promptly into the diluting solution and the pipette rinsed several times with reagent.
- Blood can be taken directly from a finger or heel puncture without use of anticoagulant.

Storage & Stability of the Specimen

Stability: 7 days at 2 -8^oC

4 days at 20- 25^oC

Warning & Precautions

1. Do not pipette the reagent with mouth as it is poisonous.
2. Keep out of reach children. In case of contact with eyes, rinse immediately with plenty of water & seek medical advice.
3. Cyanide (poison): The amount of cyanide in the Reagent Concentrate (40x) is appreciably less than the minimum lethal dose for an adult. Gaseous hydrogen cyanide will be released on contact with acids.
4. Wear suitable gloves and eye /face protection.

5. Perform the test according to the "Current Good Laboratory Practice"(cGLP) guidelines.

Assay procedure

Wave length	: 546 nm
Temperature	: 37 ^o c
Light path	: 10 mm

Pipette into cuvettes	Macro	Semi-Micro
Reagent	5ml	2.5ml
Sample	20µl	10µl
Mix & incubate for 5mins. at RT and read the absorbance against reagent Blank at 546 nm.		

Calculations

Haemoglobin in g/dl = Abs. T x 36.4

Linearity

This procedure is linear up to 20g/dl. If the value exceeds this limit, dilute the whole blood 1+1 with the normal saline NaCl (0.9%) and repeat the assay. Result × 2.

Reference Range

Infants(at birth)	14-20g/dl
Children(at 1 year)	10-14g/dl
Adult male	13-18g/dl
Adult female	11-16g/dl

"Each laboratory should check if references ranges are transferable to its own patient population & determine own preference ranges if necessary".









Quick References

Parameter	Haemoglobin
Mode	Endpoint
Wavelength	546nm
Unit	g/dl
Temperature	37 ^o C
Factor	36.4
Reaction slope	Increasing
Reagent vol.	5ml
Sample vol.	20µl
Blank	Reagent blank
linearity	20 g/dl

References

1. Drabkin DL, Austin JH; J Biol Chem 1123:51 1535
2. Henry R.J.,Clinical Chemistry – Principles and Technics, harper & Raw, 1954 pg 747

Note on symbols and marks

 Instructions for use	 Use by	 Batch number	 Manufacturer
 Invitro Diagnostic Medical Device	 Date of manufacturer	 Temperature limit	 Reference number