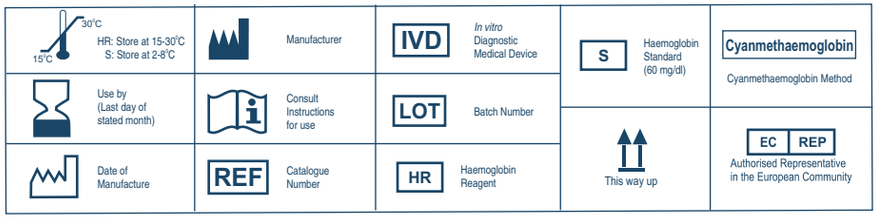
SBio HAEMOGLOBIN CONC. KIT

(Cyanmethaemoglobin Method)

(For invitro diagnostic use only)

REF	90395122
Pack Size	5 x 12.2 ml

CE



INTENDED USE

SBio Haemoglobin Conc. (25x) Kit is used for the determination of Haemoglobin in whole blood.

PRINCIPLE OF THE TEST

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

CLINICAL SIGNIFICANCE

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, 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.

PRESENTATION

5 x 12.2 ml

HR: Haemoglobin Reagent 5 x 12.2 ml

S : Haemoglobin Standard (60 mg/dl) available separately.

COMPOSITION

Phosphate Buffer 1.0 mM; pH 7.0; Potassium Ferricyanide 0.60 mM; Potassium Cyanide 0.7 mM; Detergent.

STORAGE / STABILITY

SBio Haemoglobin Conc. (25x) is stable at R.T. till the expiry date mentioned on the label.

Haemoglobin Standard is stable at 2-8°C till the expiry date mentioned on the label.

SAMPLE REQUIRED

Whole blood. Preferably fresh and collected in EDTA.

REAGENT PREPARATION

SBio Haemoglobin Conc. (25x) is concentrated.

Working Reagent: Pour the contents of 1 bottle of SBio Haemoglobin Conc. (25x) of 12.5 ml in 300 ml of deinoised water. This working reagent is stable for at least 6 months when stored at R.T. Protect from bright light and keep tightly closed.

Alternatively, for flexibility as much of working reagent may be made as and when desired by diluting SBio Haemoglobin Conc. (25x) 1:25 (1ml of SBio Haemoglobin Conc. (25x) + 24 ml of D.W.).

Precaution / Warning: Do not pipette the reagent with mouth as it is poisonous.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimen. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimen, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

PROCEDURE

Wavelength / filter : 540 nm (Hg 546 nm)

Temperature : R.T.

Light path : 1 cm

MATERIALS REQUIRED BUT NOT PROVIDED

General laboratory instrumentation like Spectrophotometer/Analyzer, Thermostatic Cuvette holder, Cuvettes, Micropipettes, Test tubes, Waterbath, Stopwatch/Timer.

Pipette into clean dry test tubes labeled as Blank (B) and Test (T):

Addition Sequence	B (ml)	T (ml)
Hemocor working reagent	5.00	5.00
Sample (well mixed whole blood)	-	0.02

Mix well and incubate at R.T. (25° C) for at least 3 minutes. Measure the absorbance of the Test Sample (Abs. T) against the Blank. The final colour is very stable.

CALCULATIONS

Haemoglobin in g/dl = Abs. T x 36.8

QUALITY CONTROL

The following process is recommended for QC during the assay of Haemoglobin. *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:

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

Limit of detection:

The limit of detection for SBio Haemoglobin Conc. (25x) is 0.1 g/dl.

Interferences:

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:

Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	12.66	1.49	13.16	1.39	25.82	2.88
Control 2	11.30	1.78	10.33	2.55	21.63	4.33

Method comparison:

Comparative studies were done to compare our reagent with another commercial Haemoglobin Conc. Assay. No significant differences were observed. Details of the comparative study are available on request.

MEASUREMENT ON COLORIMETERS

On colorimeters where the exact wavelength of 540 nm (Hg 546 nm) is not available the absorbances have to be taken on a yellow green filter.

A cyanmethaemoglobin standard (Haemoglobin Standard) available separately to be used. The absorbance of Standard (Abs.S) is taken against deionised water and noted. The test procedure remains the same as given before.

Calculations on colorimeters

$$\text{Haemoglobin in g/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times \frac{251}{1000} \times 60$$

Where

251 is the Dilution Factor i.e. Total Reaction Vol. (5.02 ml) / Sample Vol. (0.02 ml).

1000 is the Multiplication Factor to convert mgs. to grams.

60 is the Concentration of the Haemoglobin Standard in mg%.

Plotting a calibration curve

If plotting of a calibration curve is desired then pipette into five clean dry test tubes.

Dilution Factor	0.0	0.25	0.5	0.75	1.0
Addition Sequence	1 (ml)	2 (ml)	3 (ml)	4 (ml)	5 (ml)
Working Reagent	5.00	3.75	2.50	1.25	0.00
Haemoglobin Standard	0.00	1.25	2.50	3.75	5.00

Mix well and measure the absorbance of the tube Nos. 2, 3, 4 and 5 against tube No.1 which serves as a blank. Multiply the cyanmethaemoglobin concentration of the Standard (60 mg%) by 0.251 and dilution factor to get the corresponding Haemoglobin Concentration in gm% for the tube Nos. 2, 3, 4 & 5 respectively. Plot these concentrations on the horizontal (X-axis) and corresponding absorbances on the vertical axis (Y-axis). It will be a straight line passing through the origin. Read the Haemoglobin Concentration of the test on X-axis corresponding to its absorbance (Abs.T) on the Y-axis of the calibration curve.

A 3 point calibration curve if desired can also be made with only tubes No.1, 3 & 5, the rest of the procedure remains the same.

REFERENCE RANGE

Newborns : 16 - 25 g/dl

Infants : 11 - 14 g/dl

Children upto 10 yrs. : 12 - 16 g/dl

Males : 12 - 18 g/dl

Females : 12 - 16 g/dl

It is recommended that each laboratory establish its own normal range representing its patient population*.

NOTE

In case of measuring low haemoglobin concentrations, which are apparent on seeing the whole blood sample, use 0.05 ml of the whole blood as a sample, the multiplication factor, in this case, on analyzers will be 14.8 instead of 36.8.

On colorimeters, using the standard the concentration of the Std. should be taken as 24 mg% instead of 60 mg% in the calculations when using 0.05 ml of whole blood as a sample.

For convenience of a Direct Hb. Reading on a colorimeter, using the Hb. Standard, a referal chart is available on request.

The reagent may be used in several automated analyzers. Instructions are available on request. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Van Kampen E.J., Zijlstra W.G., (1961) Clin. Chem. Acta. 6 : 538.
2. Sir John V. Dacie, Lewis, S.M., Practical Haematology, 8th edition.

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EC REP

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