

**prietest™ Clinical Chemistry Reagents**

**HEMOGLOBIN - SLS**

*In vitro diagnostic test kit, for professional use only*

**INTENDED USE :** Quantitative in vitro determination of Hemoglobin in whole blood on photometric systems.

**ORDERING INFORMATION**      **Pack Size**      **Cat No.**  
1 X 1000 ml      HBLS 1000

**CLINICAL SIGNIFICANCE :**

Hemoglobin conveys dissolved gases in plasma, especially O<sub>2</sub> and CO<sub>2</sub>, and regulates cell gas exchanges. Hemoglobin also takes part in maintenance of the plasmatic buffer power. Increased levels are found in polycythaemia, congenital cyanotic heart disease, heat stroke and dehydration. Decreased levels are found in anemia resulting from deficiency of iron or folic acid, red blood hemolysis, defective globin synthesis and structural abnormalities.

**METHOD :**

Colorimetric, Sodium Lauryl Sulphate Method (Potassium Cyanide Free), End point.

**PRINCIPLE :**

Although the Cyanmethaemoglobin method recommended by ICSH is used for measurement of Hemoglobin, the presence of potassium cyanide in the formulation constitutes toxic hazard to the user & from the point of its safe disposal. Prietest Hemoglobin - SLS is free from potassium cyanide, stable, non toxic reagent.

SLS disrupts the erythrocyte membrane and brings about the change of Hemoglobin by SLS through an oxidation reaction resulting in a stable SLS-Hemoglobin complex. This complex is measured photometrically at 540 or 546 nm. Total conversion to the SLS-Hemoglobin complex is extremely rapid and SLS converts methaemoglobin fully and yields Hemoglobin concentration results that are comparable to the cyanmethaemoglobin method.

**REAGENT COMPOSITION :**

Potassium Ferricyanide      0.60 mmol/l  
Sodium Lauryl Sulphate      100 mmol/l  
Preservative & Stabilizer

**STORAGE INSTRUCTIONS AND REAGENT STABILITY :**

The reagents are stable up to the end of the indicated date of expiry on the vial label, if stored at 20°C to 25°C, protected from light and contamination is avoided.

**WARNINGS AND PRECAUTIONS :**

Take the necessary precautions for the use of laboratory reagents.

**WASTE MANAGEMENT :**

Please refer to local regulation requirements.

**REAGENT PREPARATION :**

The reagent is ready-to-use.

**MATERIAL REQUIRED BUT NOT PROVIDED :**

NaCl solution 9 g/l, General laboratory equipment, Analyser / Photometer, pipettes etc.

**SPECIMEN :**

Fresh whole blood collected in EDTA.

Discard contaminated specimens.

**ASSAY PROCEDURE :**

*Application sheets for automated systems are available on request.*

Wavelength : Hg 546 nm  
Optical path : 1 cm  
Temperature : 37° / 30° / 25°C  
Mode : End Point

Bring all the contents of the kit to Room Temperature prior to use.

Read absorbance of sample against reagent blank

Label the test tube as blank, sample, control and pipette into respective test tube the reagent, sample, control sample as per the table given below :

|                 | Blank   | Sample/ Control |
|-----------------|---------|-----------------|
| Reagent         | 1000 µl | 1000 µl         |
| Distilled Water | 10 µl   | —               |
| Sample/ Control | —       | 10 µl           |

Mix well and allow to stand at R. T. for 5 minutes then read absorbance within one hour.

**CALCULATION :**

Concentration of unknown sample (g/dl) = (Abs of Sample – Abs of Blank) X Factor

**Factor at 546 nm = 14.8**

**CONVERSION FACTOR :** Hemoglobin [g/dl] X 10 = Hemoglobin [g/L]

**CALIBRATOR :**

For the calibration of photometric systems use of the commercially available calibrator is recommended.

**QUALITY CONTROL :**

To ensure adequate quality, use of the commercially available control is recommended.

**PERFORMANCE CHARACTERISTICS :**

**MEASURING RANGE :**

The test has been developed to determine Hemoglobin concentrations within a measuring range from 1 to 20 g/dl (10 to 200 g/L).

**SPECIFICITY/ INTERFERENCE :**

No interference was observed by Ascorbic Acid up to 5 mg/dl (283.9 µmol/L), Glucose up to 500 mg/dl (27.75 mmol/L) & lipemia up to 800 mg/dl (9.12 mmol/L) Triglycerides. A list of drugs and other interfering substances with Hemoglobin determination has been reported by Young et al.

**SENSITIVITY/ LIMIT OF DETECTION :**

The lower limit of detection is 1g/dl (10 g/L).

**PRECISION :**

| Intra-assay precision n = 20 | Mean [g/dl] | SD [g/dl] | CV [%] |
|------------------------------|-------------|-----------|--------|
| Sample 1                     | 7.42        | 0.13      | 1.75   |
| Sample 2                     | 11.64       | 0.07      | 0.60   |
| Sample 3                     | 18.51       | 0.33      | 1.78   |
| Inter-assay precision n = 20 | Mean [g/dl] | SD [g/dl] | CV [%] |
| Sample 1                     | 7.56        | 0.14      | 1.85   |
| Sample 2                     | 14.45       | 0.22      | 1.52   |
| Sample 3                     | 18.67       | 0.37      | 1.98   |

**METHOD COMPARISON :**

A comparison between Robonik Prietest Hemoglobin-SLS (y) and a commercially available test (x) using 20 samples gave following results:

**Linear Regression** :  $y = 0.963x + 0.1529$  g/dl  
**Correlation Coefficient** :  $r = 0.9968$

**REFERENCE RANGE :**

**Newborns** : 16 to 25 g/dl, (160 to 250 g/L)  
**Infants** : 11 to 14 g/dl, (110 to 140 g/L)  
**Male** : 13.9 to 16.3 g/dl (139 to 163 g/L)  
**Female** : 12 to 15 g/dl (120 to 150 g/L)

*It is recommended that each laboratory should assign its own normal range.*

**LITERATURE :**

1. Van Kampen E.J. And Zijlstra W.G., Clinica. Chim. Acta., 6:538 (1961).
2. Sir John V. Dacie and S.M. Lewis., Practical Haematology, 8th edition.
3. Bauer, J.D., Hemoglobin. Clinical Chemistry, Theory, Analysis, and Correlation (Mosby ed.) 33 (1989), 513 - 517.
4. Data on file: ROBONIK (INDIA) PVT. LTD.

**MEASUREMENTS 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. Hemoglobin Standard available separately to be used. The absorbance of Standard is taken against distilled water.

**PROCEDURE :**

Pipette into clean dry test tubes labeled as Blank and Sample:

|                  | Blank   | Sample / Control |
|------------------|---------|------------------|
| Reagent          | 5000 µl | 5000 µl          |
| Distilled Water  | 50 µl   | —                |
| Sample / Control | —       | 50 µl            |

**CALCULATIONS ON COLORIMETERS :**

$$\text{Hemoglobin in g/dl} = \frac{\text{Abs. Sample} - \text{Abs. Bl.}}{\text{Abs. Std} - \text{Abs. Bl.}} \times \frac{101}{1000} \times 148$$

**Where**

- 101 : is the Dilution Factor i.e. Total Reaction Vol. (5.05 ml) / Sample Vol. (0.05 ml).
- 1000 : is the Multiplication Factor to convert mg to gram.
- 148 : is the Concentration of the Hemoglobin Standard in mg/dl.

**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) |
| Reagent           | 5.00   | 3.75   | 2.50   | 1.25   | 0.00   |
| 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/dl) by 0.251 and dilution factor to get the corresponding Hemoglobin Concentration in g/dl 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 Hemoglobin Concentration of the test on X-axis corresponding to its absorbance (Abs.S) on the Y-axis of the calibration curve.

A 3 point calibration curve if desired can also be made with only tubes No. 1, 2 & 5 the rest of the procedure remains the same.

| INSTRUMENT APPLICATION<br><b>prietest TOUCH</b> |             |
|---|-------------|
| Name : HB,                                      | Mod : END-P |
| Pri.: 546 , Sec.: 0                             |             |
| Temp: 37C , KF: 1.000                           |             |
| Vol : 500ul , Unit : g/dl                       |             |
| Lag : 5 , Read : NA                             |             |
| Blk : Y, QC : Y, Norm : Y                       |             |
| Std : N , Factor : 14.8                         |             |
| Normal HI = 16.3                                |             |
| Normal LO = 12                                  |             |
| QCNH : *  |             |
| QCNL : *  |             |
| QCABH = *                                       |             |
| QCABL = *                                       |             |
| Rgnt. Linearity : 20                            |             |
| NOTE :  |             |
| * Indicates user definable parameter.           |             |
| NA Implies Not Applicable                       |             |

| PARAMETERS FOR<br>INSTRUMENT SETTING |                |
|--------------------------------------|----------------|
| TEST NAME                            | HEMOGLOBIN-SLS |
| Reaction                             | End Point      |
| Wavelength 1                         | 546 nm         |
| Temperature                          | 37°C           |
| Zero Setting                         | Reagent Blank  |
| Factor                               | 14.8           |
| Units                                | g/dl           |
| Sample Volume                        | 10 µl          |
| Reagent Volume                       | 1000 µl        |
| Incubation Time                      | 5 minutes      |
| Reference Range                      | 12 to 16.3     |
| Reagent Linearity                    | 20             |

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**prietest TOUCH** is the Trade Mark of ROBONIK (INDIA) PVT. LTD., for Biochemistry Analyser.

**ROBONIK**  
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An ISO 13485 : 2012 Certified Company

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