

Hemoglobin

Drabkin. Colorimetric.
Liquid

Store at 2 - 8 °C

Configuration

REF	HB011
VOL	4 x 5 mL
Reagent 1	4 x 5 mL
Instrument	Universal

Intended use

Quantitative determination of hemoglobin in capillary or venous blood.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance

Hemoglobin is a porphyrin-iron (II) protein compound that transports oxygen from the lungs to the tissues where it is utilized for energy metabolism. Measurements of hemoglobin from venous or capillary blood rods are used in the detection of a variety of conditions that alter the normal hemoglobin concentration of the blood, e.g. anemia or polycythemia. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

Hemoglobin is oxidized by potassium ferricyanide into methemoglobin, which is converted into cyanomethemoglobin by potassium cyanide. The intensity of absorbance of the cyanomethemoglobin is proportional to hemoglobin concentration.

Reagent composition

Reagent 1	Dihydrogen potassium phosphate (2,00 mmol/L) Potassium ferricyanide (0,60 mmol/L) Potassium cyanide (77 mmol/L)
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Precautions

- Reagent 1: Danger. H301+H311+H331: Toxic if swallowed, in contact with skin or if inhaled. H412: Harmful to aquatic life with long lasting effects. P280: Wear eye protection, face protection, protective clothing, protective gloves. P501: Dispose of contents in an appropriate container observing applicable local regulations.
- Calibrator: The calibrator does not contain components of human origin. However, it is recommended to handle it with due care.
- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Preparation

Working reagent :

- 4,9 mL distilled water + 2 drops of reagent and mix.

Or:

- 245 mL distilled water + 5 mL of reagent and mix.

The diluted reagent (working reagent) is stable 2 months at 2-8 °C, protected from sunlight.

Hemoglobin Calibrator (HBS02) is ready to use.

Storage, stability and disposal

All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2 - 8 °C.

The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 540 nm $\geq 0,01$, the reagent should be discarded. Hemoglobin Calibrator (HBS02) is stable at 2-8 °C up to the date of expiration as specified, when stored tightly closed, protected from light and contaminations, prevented during its use.

Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided

- Spectrophotometer or colorimeter measuring at 540 nm
- Matched cuvettes 1,0 cm light path
- General laboratory equipment

Samples

Capillary or venous blood. Use anticoagulants like EDTA, heparin or oxalate. Stability 7 days at 2-8 °C.

Procedure

Make sure the reagents and samples are at room temperature.

1. Wavelength 540 nm; Temperature 15-25 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

MACRO METHOD	
For Blank	5 mL Working Reagent
For Calibrator	20 μ L Calibrator + 5 mL Working Reagent
For Sample	20 μ L Sample + 5 mL Working Reagent
Mix and wait 3 minutes at room temperature (15-25 °C). Measure the absorbance (A) against blank.	
MICRO METHOD	
For Blank	2,5 mL Working Reagent
For Calibrator	10 μ L Calibrator + 2,5 mL Working Reagent
For Sample	10 μ L Sample + 2,5 mL Working Reagent
Mix and wait 3 minutes at room temperature (15-25 °C). Measure the absorbance (A) against blank.	

Calculation

With factor ^{Note 2}:

$$\text{Hemoglobin Conc. (g/dL)} = 36,77 \times A.$$

With Calibrator:

$$\text{Hemoglobin (g/dL)} = \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Calibrator}} - A_{\text{Blank}}} \times \text{calibrator conc.} \left(\frac{g}{dL}\right)$$

Conversion Factor:

g/L = 10 x g/dL

mmol/L = 0,621 x g/dL

Quality control

Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values

Men	14 - 18 g/dL (140 - 180 g/L)
Women	12 - 16 g/dL (120 - 160 g/L)

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics

Measuring range: from 0,1 g/dL (detection limit) to 20 g/dL (linearity limit). If the obtained results are greater than 20 g/dL, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.

Precision:

	intra-assay (n=20)		inter-assay (n=20)	
	Mean (g/dL)	SD	CV (%)	CV (%)
Mean (g/dL)	8,00	15,2	7,81	15,1
SD	0,29	0,33	0,19	0,26
CV (%)	3,59	2,19	2,51	1,74

Sensitivity: 1 g/dL = 0,027 AU

Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents. The results of the performance characteristics depend on the analyzer used.

Interferences

A list of drugs and other interfering substances with hemoglobin determination has been reported by Young et al.

Notes

1. For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision.
2. The use of a calibrator is recommended instead of a factor. A Hemoglobin Calibrator is available (HBS02, 1 mL, 15 g/dL).

Bibliography

1. Franco R.S. Hemoglobin. Kaplan A et al. Clin Chem The C.V. Mosby CO. St Louis. Toronto. Princeton 1984; 1294-1296 and 418
2. Van Kampen E.J. et al. Standardization of hemoglobinometry. Clin. Chem. 1961; 6: 438-544.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press 1995
4. Young DS. Effects of diseases on Clinical Lab. Tests, 4th ed AACC 2001
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999
6. Tietz N W et al. Clinical Guide to Laboratory tests, 3rd ed AACC 1995.

