Hemoglobin Assay Kit (Colorimetric)

LS-K227-250 (250 Tests) • **Store at 4°C**

Introduction

Hemoglobin (Hb) is made of four globin chains each carrying a heme group. It is carried by red blood cells and transports oxygen from the lungs to the peripheral tissues to maintain the viability of cells. Quantitation of blood hemoglobin has been a key diagnostic parameter for various diseases such as anemia, polycythemia and dehydration.

Simple, direct and automation-ready procedures for measuring hemoglobin concentration are becoming popular in Research and Drug Discovery. This hemoglobin assay kit is based on an improved Triton/NaOH Method, in which the hemoglobin is converted into a uniform colored end product. The intensity of color, measured at 400 nm, is directly proportional to hemoglobin concentration in the sample. The optimized formulation exhibits high sensitivity and is ideal for measuring hemolysis in low hemoglobin samples (e.g. serum and plasma).

Key Features

- Sensitive and accurate. Linear detection range 0.9 - 200 mg /dL hemoglobin in 96-well plate assay.
- Simple and high-throughput. The "mix-and-read" procedure involves addition of a single working reagent and reading the optical density. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.
- Safety. Reagents are non-toxic.
- Versatility. Assays can be executed in 96-well plate or cuvette.

Applications

- Direct Assays: total hemoglobin in blood, serum, plasma, urine, etc.
- Pharmacology: effects of drugs on hemoglobin metabolism.
- Drug Discovery: HTS for drugs that modulate hemoglobin levels.

Components

Component	K227-250
	250 Tests
Reagent	50 mL
Calibrator	10 mL

Materials Not Supplied

Pipetting devices and accessories.

Procedure using 96-well plate: Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette: Cuvettes and spectrophotometer.

Storage

The kit is shipped at room temperature. Store reagent and calibrator at 4°C. Shelf life: 12 months after receipt.

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Assay Procedure

Procedure using 96-well plate

1. Blank and Calibrator. Pipette 50 µL water (Blank) and 50 µL Calibrator into wells of a clear bottom 96-well plate. Transfer 200 µL water into the Blank and Calibrator wells. The diluted calibrator is equivalent to 100 mg/dL hemoglobin.
2. Samples. Serum and plasma samples can be assayed directly (*n* = 1). Blood samples should be diluted 100-fold in distilled water (*n* = 100).

   Transfer 50 µL samples into wells (Important: avoid bubble formation during the pipetting steps). Add 200 µL Reagent to sample wells and tap plate lightly to mix.
3. Incubate 5 min at room temperature. Read OD at 390-405nm (peak 400nm).

Procedure using cuvette

1. Transfer 100 µL sample and 1000 µL Reagent into a cuvette and tap lightly to mix. Read OD 400 nm against water.
2. Transfer 100 µL Calibrator and 1000µL water to cuvette. Read OD at 400nm against water.

Calculations

Subtract blank OD (water) from the Calibrator and Sample OD values. The hemoglobin concentration of Sample is calculated as

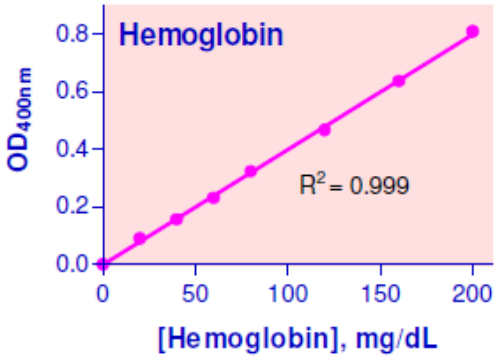
$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{CALIBRATOR} - OD_{BLANK}} \times 100 \times n \quad (mg/dL)$$

OD_{SAMPLE}, $OD_{CALIBRATOR}$ and OD_{BLANK} are OD values of the sample, the Calibrator and water. 100 mg/dL is the equivalent hemoglobin concentration of the diluted calibrator. *n* is the dilution factor (100 for blood samples).

Conversions: 1mg/dL Hb equals 0.156 µM, 0.001% or 10 ppm.

Sample Data

Hb was determined using the 96-well plate protocol. The values were 43.4± 0.4 mg/dL for rat serum, 11.2 ± 1.1 mg/dL for human plasma and 15.4 ± 0.7 g/dL for a mouse whole blood sample.



Standard Curve with Freshly Prepared Hemoglobin in 96-well plate assay

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