



Hemoglobin Assay Kit

Catalog Number KA1616

250 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

Application

- ✓ 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.

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.

Background

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. 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 substantially reduces interference by substances in the raw samples.

General Information

Materials Supplied

List of component

Component	Amount
HB Reagent	50 mL
Calibrator	10 mL

Materials Required but 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 Instruction

Store reagent and calibrator at 4°C. Shelf life: 12 months after receipt.

Precautions for Use

- Precautions

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Assay Procedure

Procedure using 96-well plate:

1. Blank and Calibrator. Pipette 50 μ L water (Blank) and 50 μ L Calibrator into wells of a clear bottom 96-well plate. Transfer 200 μ L 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 μ L samples into wells (Important: avoid bubble formation during the pipetting steps). Add 200 μ L Reagent to sample wells and tap plate lightly to mix.
3. Incubate 5 min at room temperature. Read OD at 390-405 nm (peak 400 nm).

Procedure using cuvette:

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

Data Analysis

Calculation of Results

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

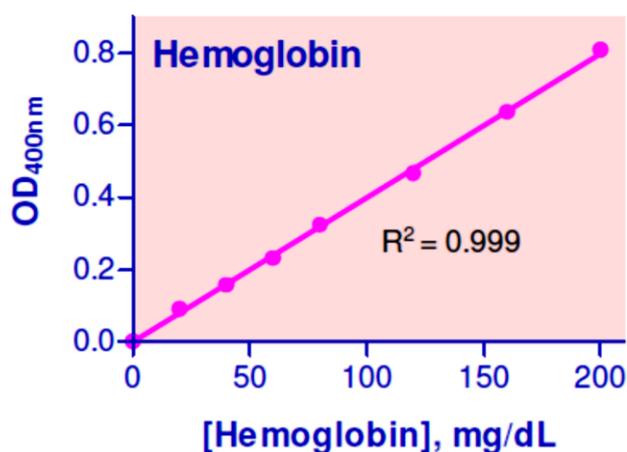
$$= \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{OD_{\text{CALIBRATOR}} - OD_{\text{BLANK}}} \times 100 \times n \text{ (mg/dL)}$$

OD_{SAMPLE} , $OD_{\text{CALIBRATOR}}$ 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.

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

✓ Example

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

Resources

References

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2. Thaker, P.H. et al (2006). Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nature Med.* 12 (8): 939-944.
3. Burne-Taney, M.J. et al (2006). Decreased capacity of immune cells to cause tissue injury mediates kidney ischemic preconditioning. *J. Immunology* 176: 7015–7020.