

Technical Manual

ChromaDazzle Hemoglobin Assay Kit

Catalogue Code: BA0043

Pack Size: 250 assays

Research Use Only

DESCRIPTION

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. The Assay Genie ChromaDazzle 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 serum, plasma, urine, etc.

Pharmacology: effects of drugs on hemoglobin metabolism.

Drug Discovery: HTS for drugs that modulate hemoglobin levels.

KIT CONTENTS (250 TESTS IN 96-WELL PLATES)

HB Reagent: 50 mL

Calibrator: 10 mL

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Procedure using 96-well plate:

- 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.*
- Samples.** Serum and plasma samples can be assayed directly ($n = 1$). 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.
- Incubate 5 min at room temperature. Read OD at 390-405nm (peak 400nm).

Procedure using cuvette:

- Blank and Calibrator.** Pipette 100 μ L water (Blank) and 100 μ L Calibrator into separate cuvettes. Transfer 1000 μ L water into the Blank and Calibrator cuvettes. *The diluted calibrator is equivalent to 100 mg/dL hemoglobin.*
- Samples.** Serum and plasma samples can be assayed directly ($n = 1$). Transfer 100 μ L samples into wells. Add 1000 μ L Reagent to sample cuvettes and tap plate lightly to mix.
- Incubate 5 min at room temperature. Read OD at 390-405nm (peak 400nm).

CALCULATION

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories.

Procedure using 96-well plate:

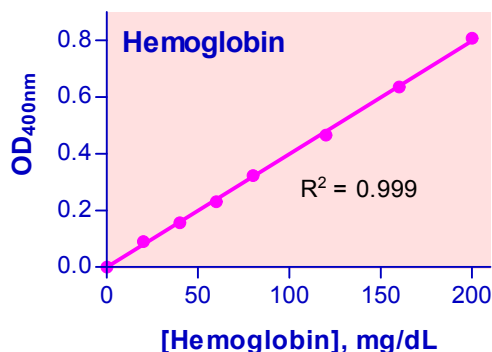
Clear-bottom 96-well plates (e.g. VWR cat# 82050-760) and plate reader.

Procedure using cuvette:

Cuvettes and spectrophotometer.

EXAMPLES

Hb was determined in duplicate 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.



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

PUBLICATIONS USING THIS KIT:

1. 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.
2. Qin, Z. et al (2007). Hyperbaric oxygen-induced attenuation of hemorrhagic transformation after experimental focal transient cerebral ischemia. *Stroke* 38:1362-1367.
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.

RELATED PRODUCTS

ChromaDazzle™ Whole Blood Hb Kit (Cat# BA0062): ideal for use with whole blood samples.

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