

Technical Manual

ChromaDazzle Whole Blood Hb Kit

Catalogue Code: BA0062

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 Whole Blood Hb 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 570 nm, is directly proportional to hemoglobin concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

KEY FEATURES

Non-hazardous. No hazardous chemicals (e.g. potassium cyanide in Drabkin's Reagent) used.

Simple and high-throughput. The "mix-and-read" procedure involves addition of a single 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.

Versatility. Assays can be executed in 96-well plate or cuvette.

High sensitivity. As low as 5 μ L sample is used. Ideal when samples are limited such as mice blood samples.

APPLICATIONS

Direct Assays: total hemoglobin in whole blood samples.

Pharmacology: effects of drugs on hemoglobin metabolism.

KIT CONTENTS (250 TESTS IN 96-WELL PLATES)

HB Reagent: 50 mL

Calibrator: 500 μ L (12.0 g/dL)

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:

Since 5 μ L samples are used, when possible perform assays in duplicate or triplicate.

1. Carefully pipette 5 μ L water (Blank), 5 μ L Calibrator and 5 μ L whole blood samples into wells of a clear bottom 96-well plate.
2. Transfer 200 μ L HB Reagent into all assay wells. Tap plate to mix.
3. Incubate 5 min at room temperature. Read OD at 570 nm (range 560-600 nm). OD values are stable for at least 60 min.

Procedure using cuvette:

1. Pipette 10 μ L water (Blank), 10 μ L Calibrator and 10 μ L whole blood samples into separate cuvettes.
2. Transfer 1 mL HB Reagent into all cuvettes. Mix well.
3. Incubate 5 min at room temperature. Read OD at 570 nm (range 560-600 nm). OD values are stable for at least 60 min.

CALCULATION

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

$$\text{Hemoglobin} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{H}_2\text{O}}}{\text{OD}_{\text{Calibrator}} - \text{OD}_{\text{H}_2\text{O}}} \times [\text{Calibrator}]$$

OD_{SAMPLE}, OD_{CALIBRATOR} and OD_{H₂O} are OD values of the sample, the Calibrator (12.0 g/dL) and water.

Conversions: 1.0 g/dL Hb is equivalent to 0.156 mM.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760) or cuvettes and plate reader or spectrophotometer.

LITERATURE

1. Tietz, NW (1976). Fundamentals of Clinical Chemistry, 2nd ed., W.B. Saunders Co., Philadelphia.
2. Thomas L (1998). Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft: 475-479.

RELATED PRODUCTS

ChromaDazzle™ Hemoglobin Assay Kit (Cat# BA0043): ideal for use with low hemoglobin samples such as serum and plasma.

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