QuantiChrom™ Hemoglobin Assay Kit (DIHB-250)

Colorimetric Determination of Total Hemoglobin at 400 nm

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. BioAssay Systems' QuantiChrom™ hemoglobin assay kit is based on an improved cyanohemiglobin 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.

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.

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 cuvet.

KIT CONTENTS (250 tests in 96-well plates)

Reagent: 50 mL
Standard: 10 mL (= 200 mg/dL hemoglobin)

Storage conditions. Store reagent and standard at 4°C. Shelf life: 12 months.

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:

1. *Blank and Standard*. Pipette 50 µL water (Blank) and 50 µL Standard into wells of a clear bottom 96-well plate. Transfer 200 µL water into the blank and standard wells.

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.

3. Add 200 µL Reagent to sample wells and tap plate lightly to mix.

4. Incubate 5 min at room temperature. Read OD at 380-420nm (peak 400nm). Signal is stable for at least 2 hours.

Procedure using cuvette:

1. Transfer 100 µL sample and 1000 µL Reagent into a cuvet and tap lightly to mix. Read OD at 380-420nm (peak 400 nm) against water.

2. Transfer 100 µL Standard and 1000µL water to cuvet. Read OD at 400nm against water.

CALCULATION

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

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STD} - OD_{BLANK}} \times 200 \times n \quad (mg/dL)$$

OD_{SAMPLE}, OD_{STD} and OD_{BLANK} are OD values of the sample, the Standard and water. *n* is the dilution factor (100 for blood samples).
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:

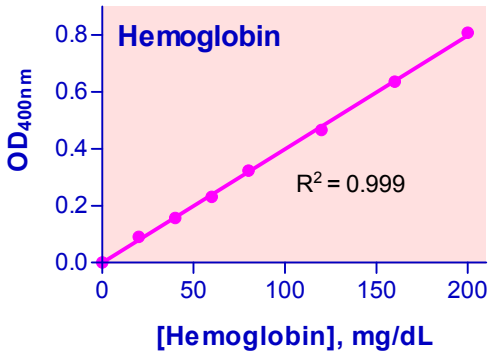
Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Cuvets and spectrophotometer.

EXAMPLES

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.



Calibration curve in 96-well plate

LITERATURE

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3. Van Kampen EJ, Zijlstra WG (1965). Determination of hemoglobin and its derivatives. Adv Clin Chem. 8:141-187.