

Technical Bulletin

Whole Blood Hemoglobin (Hb) Kit

Catalog Number MAK455

Product 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-suitable procedures for measuring hemoglobin concentration are becoming common in research and drug discovery. The Whole Blood Hemoglobin (Hb) Kit is based on an improved aqueous alkaline 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 the hemoglobin concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

The kit is suitable for the quantitative determination of hemoglobin (Hb) in whole blood.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

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|--|--------|
| • Hb Reagent
Catalog Number MAK455A | 50 mL |
| • Calibrator (12.0 g/dL)
Catalog Number MAK455B | 500 µL |

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge Calibrator prior to opening.

Procedure

All samples should be run in duplicate.

1. Carefully pipette 5 µL of purified water (Blank), 5 µL Calibrator and 5 µL whole blood (Sample) into separate wells of a clear 96-well plate.
2. Add 200 µL of HB Reagent into all assay wells. Tap plate to mix.
3. Incubate the plate for 5 minutes at room temperature.

Measurement

Read the optical density (OD) at 570 nm.
OD values are stable for at least 60 minutes.

Results

The hemoglobin concentration of the Sample is calculated as follows:

Hemoglobin (g/dL) =

$$\frac{OD_{Sample} - OD_{Blank}}{OD_{Calibrator} - OD_{Blank}} \times 12.0 \text{ g/dL}$$

where

OD_{Sample} = Optical density at 570 nm of the Sample

OD_{Blank} = Optical density at 570 nm of the water Blank

OD_{Calibrator} = Optical density at 570 nm of the Calibrator

12.0 g/dL = Concentration value of the Calibrator

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

References

1. Murata, Y., et al., Iron uptake mediated by the plant-derived chelator nicotianamine in the small intestine. *J. Biol. Chem.*, **296**, 100195 (2020).
2. Ward, E., et al., Soy protein is an efficacious alternative to whey protein in sorghum-soy fortified blended foods in rats. *Curr. Dev. Nutr.*, **4(8)**, nzaa115 (2020).



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