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Product Information

Hemoglobin Assay Kit

Catalog Number **MAK115**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Hemoglobin (Hb) is an iron-containing metalloprotein that serves as the primary means of oxygen transport in vertebrates. Hemoglobin is primarily found in red blood cells where it makes up to 97% of the cell's dry content. Hemoglobin can also be found in other tissues where it serves as an antioxidant. Alterations in blood hemoglobin levels occurs in many diseases such as anemia and polycytheima.

The Hemoglobin Assay kit provides a simple and direct procedure for measuring hemoglobin levels in a variety of samples such as blood, serum, plasma, and urine. This assay is based on the improved Triton®/NaOH method in which hemoglobin is converted to a colorimetric product measured at 400 nm. This assay has a linear detection range between 0.9–200 mg/dL in the 96 well plate assay.

Components

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

Reagent Catalog Number MAK115A	50 mL
Calibrator Catalog Number MAK115B	10 mL

Reagents and Equipment Required but Not Provided.

- Spectrophotometric multiwell plate reader
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped at room temperature. Storage at 2–8 °C, protected from light, is recommended.

Procedure

Sample Preparation

Serum, plasma, and urine sample can be assayed directly.

Blood samples should be diluted 100-fold in water.

Assay Reaction

1. Add 50 µL of water (Blank) and 50 µL of the Calibrator into wells of a clear bottom 96 well plate. Add 200 µL of water into the Blank and Calibrator. The diluted calibrator is equivalent to 100 mg/dL hemoglobin.

2. Transfer 50 µL of samples into wells. Add 200 µL of Reagent to sample wells and tap plate lightly to mix.

3. Incubate 5 minutes at room temperature.

4. Measure the absorbance at 400 nm (A_{400}).

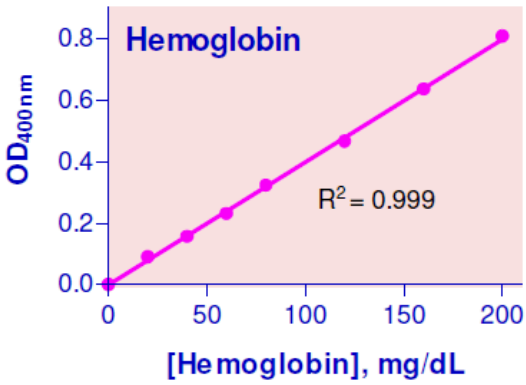
Note: This procedure can be adapted for use in cuvettes. Transfer 100 µL of sample and 1,000 µL of Reagent into a cuvette and tap lightly to mix. Measure the absorbance at A_{400}. Transfer 100 µL of Calibrator and 1,000 µL of water to a cuvette. Measure the absorbance A_{400}. Blank by reading the A_{400} of water.

Calculations
Concentration of Hemoglobin

$$= \frac{(A_{400}\ \text{sample}) - (A_{400}\ \text{blank})}{(A_{400}\ \text{calibrator}) - (A_{400}\ \text{blank})} \times 100\ \text{mg/dL} \times \text{df}$$

100 mg/dL = concentration of the diluted calibrator
df = dilution factor (for example, 100 for the blood samples)

Conversion factors for Hemoglobin:
1 mg/dL = 0.156 µM, 0.001%, or 10 ppm.



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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Presence of interfering substance in the sample	If possible, dilute sample further
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Samples measured at incorrect wavelength	Check the equipment and filter settings

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