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ab272533
Hemoglobin Assay Kit

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Hemoglobin Assay Kit datasheet:
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For quantitative determination of Hemoglobin concentration in biological samples.

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1. Overview

Hemoglobin Assay Kit is a simple, direct and automation-ready procedure for measuring hemoglobin concentration. This assay 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 substantially reduces interference by substances in the raw samples.

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.

2. Protocol Summary

Prepare all reagents and samples as instructed

⇩

Add Samples, Blank and Standard to appropriate wells.

⇩

Add Reagent to samples and add H_2O to Blank and Standard.

⇩

Incubate for 5 minutes at room temperature.

⇩

Read absorbance at 400 nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent	50 mL	+4°C
Calibrator	10 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Distilled H2O
- Multi-channel pipette
- 1.5 mL tubes
- 1.5 mL centrifuge
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 390-405 nm (peak absorbance is at 400 nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate reagent to room temperature (18-25°C) prior to use.
- Reagent comes as is ready to use.
- The kit contains enough reagents for 250 assays.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare diluted standards immediately prior to use.

Diluted Standard:

10.1.1 Add 50 µL of Calibrator and 200 µL of H_2O into wells of a 96-well plate.

- The diluted Standard is equivalent to 100 mg/dL Hemoglobin.

11. Sample Preparation

Sample treatment:

11.1.1 Serum and plasma samples can be assayed directly (*n*=1).
11.1.2 Blood samples should be diluted 100-fold in distilled water (*n*=100).

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Procedure using 96-well plate:

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

12.1.2 Samples. Serum and plasma samples can be assayed directly (n = 1). Transfer 50 µL samples into wells. Add 200 µL Reagent to sample wells and tap plate lightly to mix.

12.1.3 Incubate for 5 min at room temperature.

12.1.4 Read OD at 380-420 nm (peak 400 nm).

ΔNote: avoid bubble formation during the pipetting steps.

Procedure using cuvette:

12.1.5 Blank and Calibrator. Pipette 100 µL water (Blank) and 100 µL Calibrator into separate cuvettes. Transfer 1000 µL water into the Blank and Calibrator cuvettes. The diluted calibrator is equivalent to100 mg/dL hemoglobin.

12.1.6 Samples. Serum and plasma samples can be assayed directly (n = 1). Transfer 100 µL samples into wells. Add 1000 µL Reagent to sample cuvettes and tap plate lightly to mix.

12.1.7 Incubate 5 min at room temperature. Read OD at 390-405nm (peak 400nm).

13. Calculations

13.1.1 Subtract Blank OD (H_2O) from the calibrator and Sample OD values.

13.1.2 The total Hemoglobin concentration of Sample is calculated as

$$\frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \; X \; 100 \; X \; n \; (mg/dL)$$

OD_{SAMPLE} is OD values of sample.

OD_{BLANK} is OD values of H_2O.

$OD_{STANDARD}$ is OD values of the calibrator.

n is the dilution factor

Δ **Note:** 1mg/dL Hb equals 0.156 µM, 0.001% or 10 ppm.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

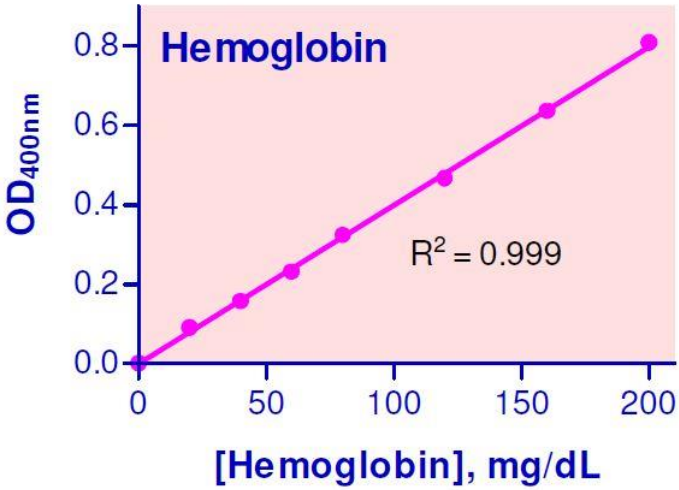

Figure 1. Example of Hemoglobin Assay Kit standard curve.

15. Notes

Technical Support

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