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.

This product is for research use only and is not intended for diagnostic use.
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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 H₂O 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

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>50 mL</td>
<td>+4°C</td>
</tr>
<tr>
<td>Calibrator</td>
<td>10 mL</td>
<td>+4°C</td>
</tr>
</tbody>
</table>
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 H₂O 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 Add 250 µL of H₂O to wells of a 96-well plate (Blank).
12.1.2 Take Diluted calibrator as prepared in 10.1.1.
12.1.3 Add 50 µL of each sample to separate wells.

**Note:** avoid bubble formation during the pipetting steps.
12.1.4 Add 200 µL of the Reagent to each sample and tap plate lightly to mix.
12.1.5 Incubate for 5 min at room temperature.
12.1.6 Read OD at 380-420 nm (peak 400 nm).

Procedure using cuvette:
12.1.7 Add 100 µL sample and 1000 µL Reagent to a cuvette and tap lightly to mix.
12.1.8 Read OD at 380-420nm (peak 400 nm) against H₂O.
12.1.9 Add 100 µL of Standard and 1000µL H₂O to cuvette.
12.1.10 Read OD at 400nm against H₂O.
13. Calculations

13.1.1 Subtract Blank OD (H₂O) from the calibrator and Sample OD values.

13.1.2 The total Hemoglobin concentration of Sample is calculated as

\[
\frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 100 \times n \text{ (mg/dL)}
\]

- \(OD_{SAMPLE}\) is OD values of sample.
- \(OD_{BLANK}\) is OD values of H₂O.
- \(OD_{STANDARD}\) is OD values of the calibrator.
- \(n\) is the dilution factor

**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.
Technical Support

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