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ab272534

Heme Assay Kit

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Heme Assay Kit datasheet:

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For quantitative determination of Heme concentration in biological samples.

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

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1. Overview

Heme Assay Kit (ab272534) is a simple, direct and automation-ready procedure for measuring heme concentration. This assay is based on an improved aqueous alkaline solution method, in which the heme is converted into a uniform colored form. The intensity of color, measured at 400 nm, is directly proportional to the heme concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples and exhibits high sensitivity.

Sensitive and accurate: Linear detection range 0.6 – 125 μM heme 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 cuvette.

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. 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.
- The kit contains enough reagents for 250 assays.

Reagents are supplied ready to use.

10. Standard Preparation

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

See Section 12 for use of the Calibrator.

11. Sample Preparation

Serum and plasma samples:

Serum and plasma can be assayed directly (dilution factor =1).

Blood samples:

Blood samples should be diluted 100-fold in distilled water (dilution factor =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.

12.1 Procedure using 96-well plate:

12.1.1 Add 50 μL of H_2O to appropriate wells(Blank).

12.1.2 Add 50 μL of Calibrator to appropriate wells.

12.1.3 Add 50 μL of each sample to appropriate wells.

Δ Note: avoid bubble formation during the pipetting steps.

12.1.4 Add 200 μL water to the blank and Calibrator wells (Calibrator = 62.5 μM heme).

12.1.5 Add 200 μL of Reagent to each sample and tap plate lightly to mix.

12.1.6 Incubate for 5 min at room temperature.

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

Δ Note: Use 96-well clear, flat-bottom plates.

12.2 Procedure using cuvette:

12.2.1 Add 100 μL sample and 1000 μL Reagent to a cuvette and tap lightly to mix.

12.2.2 Read OD at 380-420nm (peak 400 nm) against H_2O .

12.2.3 Add 100 μL of Calibrator and 1000 μL H_2O to cuvette.

12.2.4 Read OD at 380-420nm (peak 400 nm) against H_2O .

13. Calculations

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

13.1.2 The total heme concentration of Sample is calculated as

$$[\text{Heme}] = \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{OD_{\text{STANDARD}} - OD_{\text{BLANK}}} \times 62.5 \times n \text{ } (\mu\text{M})$$

OD_{SAMPLE} is OD value of sample.

OD_{BLANK} is OD value of H₂O.

OD_{STANDARD} is OD value of the Standard.

n is the dilution factor (e.g. 100 for blood samples diluted 100-fold in water).

Conversions: 1mg/dL Heme equals 15.3 μM, 0.001% or 10 ppm.

14. Notes

Below is a dilution series for freshly prepared Heme for visualization of the assay linearity - please note standard curve generation is not required for this assay.

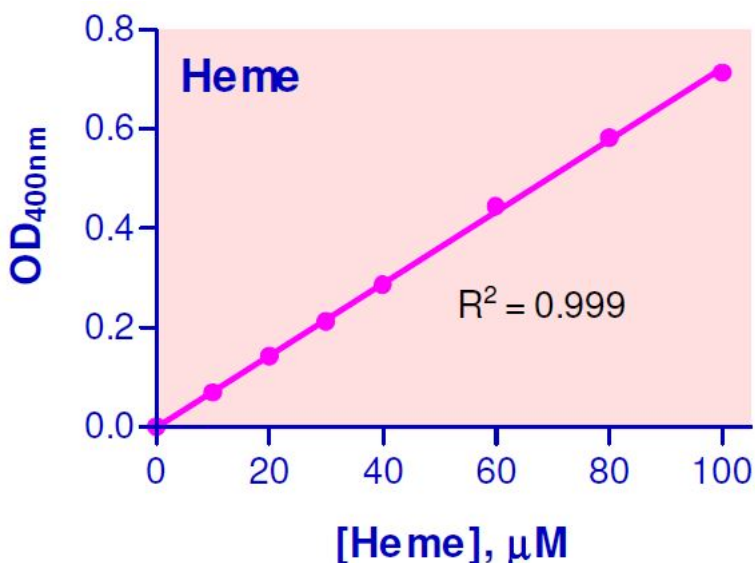


Figure 1. Standard curve with freshly prepared Heme

Typical values:

Heme was determined using the 96-well plate protocol. The values were $27.3 \pm 0.2 \mu\text{M}$ for rat serum, $7.8 \pm 0.4 \mu\text{M}$ for human plasma and $11.2 \pm 0.2 \text{ mM}$ for a mouse whole blood sample.

Technical Support

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