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Product Information

Heme Assay Kit

Catalog Number **MAK316**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Heme is a member of the porphyrin family. It is synthesized in both mitochondria and the cytoplasm, and is a key prosthetic group for various essential proteins such as hemoglobin, cytochromes, catalases and peroxidases. Free heme, which can be released from hemoglobin following hemolysis, is pro-inflammatory and contributes to iron-derived reactive oxygen species. Heme determination is widely practiced by researchers of various blood diseases.

The Heme Assay Kit provides a simple and high-throughput adaptable assay to measure total heme concentration in a variety of biological samples such as blood, plasma, serum, urine, and heme carrying enzymes. This assay is based on an aqueous alkaline solution method, in which the heme is converted into a uniform colored form, producing a colorimetric (400 nm) result, directly proportional to the heme concentration in the sample. The optimized formulation reduces interference and exhibits high sensitivity.

One mg/dL heme equals 15.3 μM, 0.001%, or 10 ppm. The kit has a linear detection range of 0.6–125 μM heme in a 96 well format.

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

Heme Reagent Catalog Number MAK316A	50 mL
Heme Calibrator Catalog Number MAK316B	10 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate or cuvettes – It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader

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.

Preparation Instructions
Briefly centrifuge vials before opening.

Storage/Stability
The kit is shipped at room temperature. Storage at 2–8 °C is recommended.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples and standards.

Sample Preparation
Serum and plasma can be assayed directly. Blood samples should be diluted 100-fold in ultrapure water.

Assay Reaction using 96 well plates
1. Add 50 μL of water (blank) and 50 μL of Heme Calibrator into wells of a 96 well plate. Add 200 μL of water into each of the blank and Heme Calibrator reaction wells. (The diluted Heme Calibrator is equivalent to 62.5 μM heme).
2. Add 50 μL of samples into wells. Add 200 μL of Heme Reagent to each sample well. Tap plate lightly to mix.
3. Incubate plate for 5 minutes at room temerature.
4. Measure the absorbance at 400 nm (A_{400})

Assay Reaction using cuvettes
1. Add 100 μL of sample and 1,000 μL of Heme Reagent to a cuvette. Tap lightly to mix. Measure the absorbance at 400 nm (A_{400}) against water.
2. Add 100 μL of Heme Calibrator and 1,000 μL of water to a cuvette. Tap lightly to mix. Measure the absorbance at 400 nm (A_{400}) against water.

Results

The Total Heme Concentration of a sample may be determined by the following equation:

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$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{CALIBRATOR} - OD_{BLANK}} \times 62.5 \times n \ (\mu M)$$

n = Dilution factor (i.e. 100 for blood samples.)

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	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	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range