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Product Information

Hemin Assay Kit

Catalog Number **MAK036**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Hemin, the oxidized version of heme, is an iron-containing prosthetic group for a diverse group of proteins. Free heme, which can be released from hemoglobin following hemolysis, is pro-inflammatory and contributes to iron-derived reactive oxygen species. Free hemin levels may be upregulated in various pathological conditions and can contribute to various inflammatory conditions including vascular disorders, renal failure, and immune-mediated disorders.

In this assay, Hemin concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm) product, proportional to the hemin present.

This kit is suitable for use with cell and tissue culture supernatants, urine, plasma, serum, fecal material, media, and other biological fluids.

Components

The kit is sufficient for 100 assays in 96 well plates.

Hemin Assay Buffer Catalog Number MAK036A	25 mL
Hemin Probe, in DMSO Catalog Number MAK036B	0.2 mL
Enzyme Mix Catalog Number MAK036D	1 vl
Hemin Substrate Catalog Number MAK036E	1 mL
Hemin Standard, 1 nmole Catalog Number MAK036F	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Dimethyl Sulfoxide (Catalog Number D2650 or equivalent)

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. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Hemin Assay Buffer – Allow buffer to come to room temperature before use.

Hemin Probe – Thaw at room temperature to melt solution prior to use. Aliquot and store protected from light and moisture at 2–8 °C.

Enzyme Mix – Reconstitute in 0.5 mL of Hemin Assay Buffer. Mix well by pipetting, then aliquot and store protected from light and moisture at –20 °C.

Hemin Substrate – Ready-to-use as supplied. Store at 2–8 °C.

Hemin Standard – Reconstitute with 100 µL of DMSO to make a 10 µM solution. Mix well by pipetting, then aliquot and store, protected from light and moisture, at 2–8 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at 2–8 °C (except where indicated), protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Hemin Standards for Colorimetric Detection
Dilute 10 µL of the 10 µM Hemin Standard Solution with 990 µL of Hemin Assay Buffer to prepare a 100 nM standard solution. Dilute 100 µL of the 100 nM solution with 900 µL of Hemin Assay Buffer to prepare a 10 nM (10 fmole/µL) standard solution. Add 0, 4, 8, 12, 16, and 20 µL of the 10 nM Hemin standard solution into a 96 well plate, generating 0 (blank), 40, 80, 120, 160, and 200 fmole/well standards. Add Hemin Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10–100 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold Hemin Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material. Samples should typically be diluted 100 to 1,000-fold, depending on the hemin content.

Serum and other liquid samples can be directly added to the wells.

Bring samples to a final volume of 50 µL with Hemin Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Hemoproteins present in the sample can generate background. To control for background, include a blank sample for each sample by omitting the Enzyme Mix in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).
   Note: The proper order of addition of the following reaction mix components is critical. Prepare the reaction mix by adding the components in the order listed in Table 1.

Table 1.
Reaction Mixes

Reagent	Sample Blank	Samples and Standards
Enzyme Mix	–	3 µL
Hemin Substrate	2 µL	2 µL
Hemin Assay Buffer	46 µL	43 µL
Hemin Probe	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 10–30 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 570 nm (A_{570}).
   Note: It is essential to measure the absorbance in kinetic mode, the highest standard should have an A_{570} in the range of 0.7–1.3.

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Hemin Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Hemin standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Hemin present in the sample may be determined from the standard curve.

Concentration of Hemin

$$S_a/S_v = C$$

S_a = Amount of Hemin in unknown sample (fmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of Hemin in sample

Hemin molecular weight: 652 g/mole

Sample Calculation

Amount of Hemin (S_a) = 58.4 fmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Hemin in sample

$$58.4 \text{ fmole}/50\ \mu\text{L} = 1.168 \text{ fmole}/\mu\text{L}$$

$$1.168 \text{ fmole}/\mu\text{L} \times 652 \text{ fg/fmole} = 761.5 \text{ fg}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice 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 Master Reaction Mix before each 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 Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	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

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