Product Information

Hemin Colorimetric Assay Kit

I. Kit Contents:

Components	K2215-100 100 assays	Cap Color	Part Number
Hemin Assay Buffer	25 ml	WM	K2215-C-1
Hemin Probe (in DMSO)	0.2 ml	Red	K2215-C-2
Enzyme Mix	1 vial	Green	K2215-C-3
Hemin Substrate	1 ml	Blue	K2215-C-4
Hemin Standard (1 nmol; lyophilized)	1 vial	Yellow	K2215-C-5

II. Introduction:

Hemin is an iron-containing porphyrin and comes from the breakdown of hemin-containing proteins such as myoglobin and hemoglobin. Free hemin can be detected in various body fluids (such as csf, saliva and urine) under various pathological states and exists at a very minute concentrations ($< 1\mu M \approx$ 650 ng/ml) in cells. Hemin is involved in induction of microsomal hemin oxygenase-1 and inhibition of nonspecific δ-aminolevulinate synthase expression. Hemin also stimulates growth of oral bacteria involved in gingivitis. When found in the urine or feces, it is indicated the possible pathological conditions.

The Hemin Colorimetric Assay Kit provides a highly sensitive, fast and convenient way for detection of hemin levels in various samples based on colorimetric method. The assay utilizes peroxidase activity in the presence of hemin to cause the conversion of a colorless probe to a strongly colored ($\lambda = 570$) compound. Trace amounts of Hemin can be quantitated in the 5 - 160 pg (10 - 250 fmol) range.

III. Reagent Preparation and Storage Conditions:

Probe: Warm at 37℃ for 1 - 2 min to completely melt before use. Mix well, store at 4℃, protect from light and moisture. Use within two months.

Enzyme Mix: Dissolve in 0.5 ml Hemin Assay Buffer, mix well. Store at -20℃.

Hemin Substrate: Ready to use as supplied. Store at 4℃; Use within two months.

Hemin Standard: Dissolve with 100 µl DMSO to make a 10 µM solution. Store at 4℃; Use within two months.

IV. Hemin Assay Protocol:

1. Standard Curve Preparations: Immediately before use, dilute the 10 µM Hemin Standard to 100 nM by adding 10 µl of the Standard to 990 µl of Hemin Assay Buffer, mix well. Dilute further to 10 nM (= 10 fmol/µl) by adding 100 µl to 900 µl Hemin Assay buffer. Add 0, 4, 8, 12, 16, 20 µl into a series of wells. Adjust volume to 50 µl/well with Hemin Assay Buffer to generate 0, 40, 80, 120, 160, 200 fmol/well of the Hemin Standard.

2. Sample Preparations: Depending upon the hemin content, samples should be diluted typically 100 to 10,000 fold and added at about 1 - 10 µl of diluted sample per well. Samples can be assayed without any prior treatment. Hemin concentration in samples may have a wide range. For different sample types, we suggest using ~ 0.04 µl serum sample, ~ 50 µg of feces, ~ 1 - 5000 cultured cells or ~ 0.05 µl urine. Place diluted samples directly in wells and adjust well volumes to 50 µl with Hemin Assay Buffer in a 96-well plate. We suggest using several doses of your sample to ensure the readings are within the standard curve range.

The presence of hemoproteins may interfere with the assay although in our experience, the very high dilution factor reduces the concentration of any such proteins to undetectable levels. You may do a sample background control without the Enzyme Mix in the reaction, then subtract the sample background from your sample readings.

3. Reaction Mix Preparation: NOTE: The proper order of addition of the following components is critical.

Immediately before use, mix enough reagent for the number of assays performed. For each well, prepare a 50 µl Reaction Mix containing the following components in the following order

Enzyme Mix	3 µl
Hemin Substrate	2 µl
Assay Buffer	43 µl
Probe	2 µl

4. Add 50 µl of the Reaction Mix to each well containing the Hemin Standard or test samples, mix well.

5. Incubate the reaction for 30-60 min at room temperature, protect from light. As this is an enzyme activity assay, it is important to incubate and measure your samples at the same time and under exactly the same conditions as the standards. The 30-60 minute incubation time has been selected as the best compromise between linearity, speed and sensitivity. It is advantageous to read the assay in kinetic mode (as shown below), observing the color development as it proceeds, using measurement data in the range of 0.7 - 1.3 OD for the highest standard (200 fmol).

6. Measure the OD at 570 nm.

7. Calculation: Correct background by subtracting the value derived from the 0 Hemin control from all sample and standard readings (Note: The background reading may be significant and must be subtracted from sample readings). Plot standard curve pmol/well vs. OD 570 nm readings. Then apply the sample readings to the standard curve to get Hemin amount in the sample wells (Hy). Calculate the Hemin concentrations in the test samples as follows:

C (fmol/µl or nM) = Hy/Sv x Ds

Where: Hy is the amount of Hemin (fmol) of your sample from standard curve.

Sv is the sample volume (µl) added into the sample well.

Ds is the dilution factor of the sample, i.e. 100 or 10,000.

Hemin molecular weight: 652. Hemin concentration in your sample can be expressed as pmol/ml, ng/ml, µg/dL or µM (µmol/liter); 1 µM = 1 nmol/ml = 652 ng/ml.

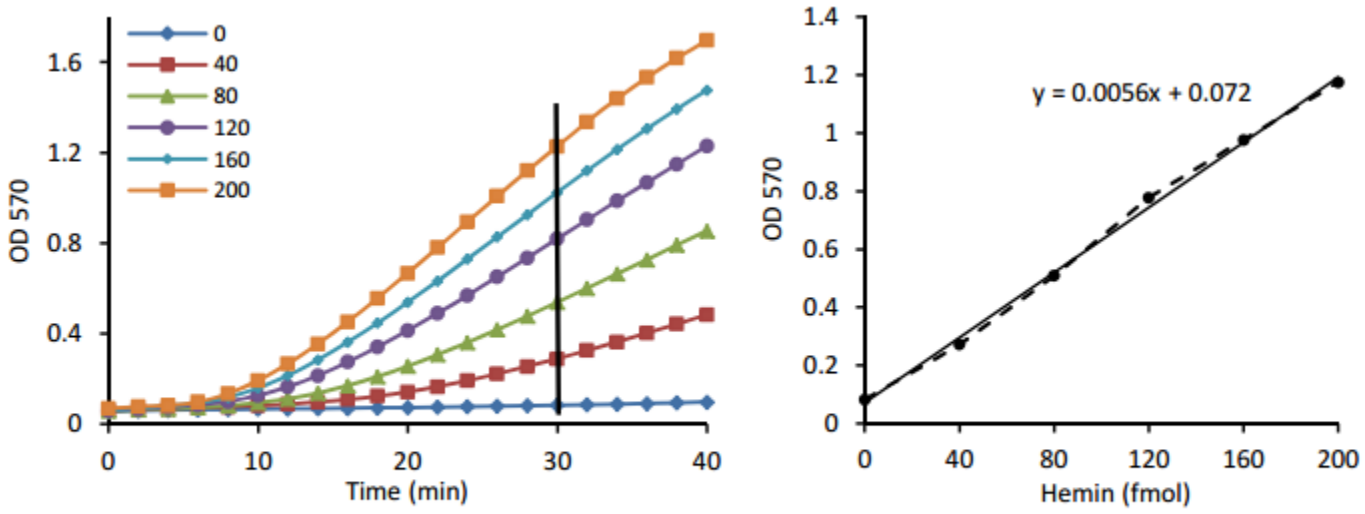

Fig. 1 A: Time course of development of color and B: Standard Curve at 30 minutes for Hemin standard as performed according to this protocol

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com