Hemin Assay Kit (Colorimetric)

LS-K615-100 (100 Tests) • **See Storage Conditions Below**

Introduction

Free hemin results from the breakdown of hemin-containing proteins such as hemoglobin and myoglobin. It can be detected in various body fluids such as saliva, urine and CSF under various pathological states. Free hemin exists in cells at very minute concentrations (< 1 µM ≈ 650 ng/ml) exerting regulatory functions such as repression of nonspecific δ-aminolevulinate synthase expression and induction of microsomal hemin oxygenase-1. Hemin can stimulate growth of oral bacteria involved in gingivitis and is an indicator of possible pathological conditions when found in the urine or feces. LSBio's Hemin Assay Kit utilizes peroxidase activity in the presence of hemin to provide a simple, sensitive assay which causes the conversion of a colorless probe to a strongly colored (λ = 570 nm) compound. Trace amounts of Hemin can be quantified in the 5-160 pg (10 - 250 fmol) range.

Components

Component	K615-100 100 Tests	Cap Code
Hemin Assay Buffer	25 ml	WM
Hemin Probe (in DMSO)	0.2 ml	Red
Enzyme Mix	1 vial	Green
Hemin Substrate	1 ml	Blue
Hemin Standard (1 nmol; Lyophilized)	1 vial	Yellow

Materials Not Supplied

- 96-well plate
- DMSO

Storage Conditions and Reagents Preparation

- Probe: Warm at 37°C for 1-2 min to completely melt before use. Mix well, store at 4°C, protect from light and moisture. Use within two months.
- Enzyme Mix: Dissolve in 0.5 ml Hemin Assay Buffer, mix well. Store at -20°C.
- Hemin Substrate: Ready to use as supplied. Store at 4°C; Use within two months.
- Hemin Standard: Dissolve with 100 µl DMSO to make a 10 µM solution. Store at 4°C; Use within two months.

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LSBio
LifeSpan BioSciences, Inc.

Assay Procedure

1. Standard Curve Preparation: 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.

   1. Enzyme Mix: 3 µl
   2. Hemin Substrate: 2 µl

   Incubate for 2 minutes at room temperature before adding the following components:

   3. Assay Buffer: 43 µl
   4. 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 10-30 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 10-30 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.

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Sample Data

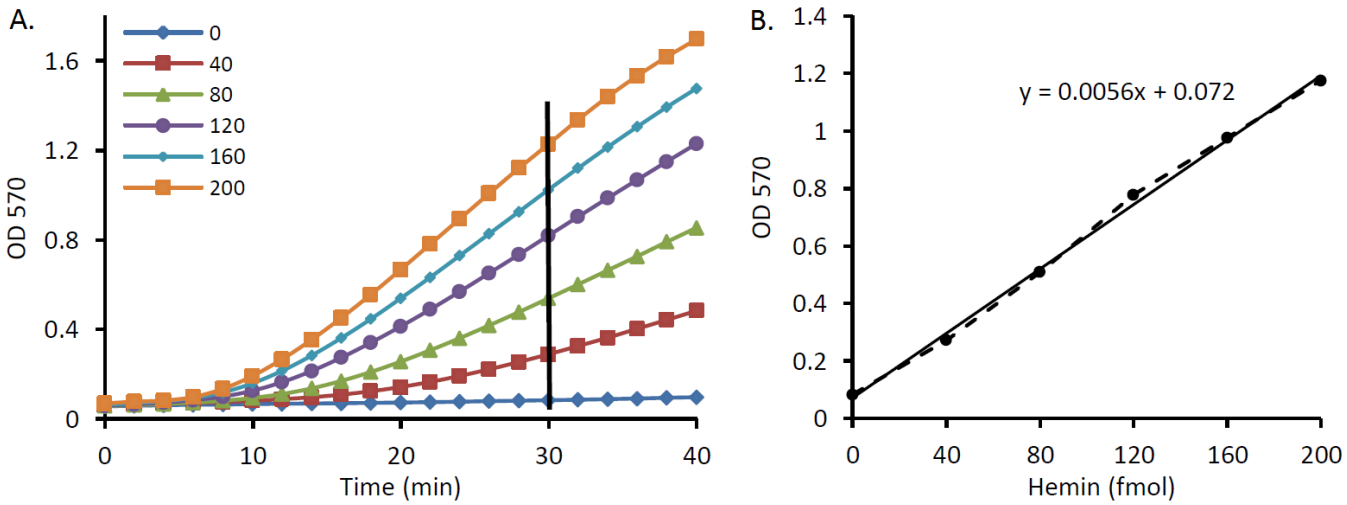

Fig. 1 A.: Time course of development of color and B.: Standard Curve at 30 minutes for Hemin standard as performed according to this protocol

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