For research use only

Hemin Assay Kit

(Catalog #K672-100; 100 assays; Store kit at -20°C)

I. 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 a very minute concentrations (< 1µM ≈ 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. BioVision's Hemin Assay Kit utilizes peroxidase activity in the presence of hemin to provide a simple, exquisitely sensitive assay which causes the conversion of a colorless probe to a strongly colored (λ = 570) compound. Trace amounts of Hemin can be quantitated in the 5-160 pg (10-250 fmol) range.

II. Kit Contents:

Components	100 assays	Cap Code	Part Number
Hemin Assay Buffer	25 ml	WM	K672-100-1
Hemin Probe	1 Vial	Red	K672-100-2
DMSO (anhydrous)	400 µl	Brown	K672-100-3
Enzyme Mix	1 Vial	Green	K672-100-4
Hemin Substrate	1 ml	Blue	K672-100-5
Hemin Standard (1 nmol)	lyophilized	Yellow	K672-100-6

III. Reagent Preparation and Storage Conditions:

Probe: Dissolve with 220 µl of DMSO (provided, need to warm up >18°C to become liquid) before use. Mix well, store at –20°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: Dissolve with 100 µl DMSO to give a 10 µM solution. Stable for 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, 5, 10, 15, 20, 25 µl into a series of wells. Adjust volume to 50 µl/well with Hemin Assay Buffer to generate 0, 50, 100, 150, 200, 250 fmol/well of the Hemin Standard.
2. **Sample Preparations:** Depending upon 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 to use ~ 0.04 µl serum sample, or ~50 µg of feces, or ~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.

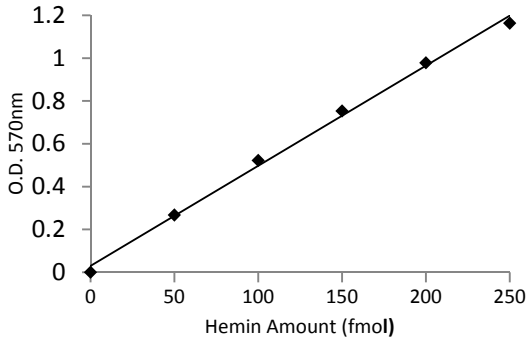
   *The presence of hemoproteins may interfere with the assay although in our experience, the very high dilution factor reduces the concentration of any such proteins to undetectable levels. You may do a sample background control without the Enzyme Mix in the reaction, then subtract the sample background from your sample readings.
3. **Enzyme Mix Addition:** Add 4 µl of Enzyme Mix to each well containing the Hemin Standard or test samples, mix well.
4. **Reaction Mix Preparation:** Immediately before use, mix enough reagents for the number of assays performed. At this time, dilute the substrate 1:10 by adding 100 µl of substrate to 900 µl of Hemin Assay Buffer. For each well, prepare a total 46 µl Reaction Mix containing the following components.

   2 µl Probe
   2 µl Substrate
   42 µl Assay Buffer
5. Add 46 µl of the Reaction Mix to each well containing the Hemin Standard or test samples, mix well.
6. Incubate the reaction for 30 minutes at room temperature, protect from light.
7. Get your plate reader ready during the incubation. Measure the O.D. at 570 nm .
8. **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. O.D. 570 nm readings. Then apply the sample readings to the standard curve to get Hemin amount in the sample wells (Hy).

   The Hemin concentrations in the test samples:

   C = Hy/Sv * Ds (fmol/µl or nM)

   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.



V. Related Products:

Lactate Assay Kit	Glutathione Assay Kit
Ascorbic acid Assay Kit	Free Fatty Acid Assay Kit
NAD(P)/NAD(P)H Assay Kit	ATP/ADP Assay Kit
Cell Proliferation Assay Kit	Cytotoxicity Assay Kit