Heme Assay Kit

Heme Assay kit is a detection kit for the quantification of Heme Content in blood, serum, plasma, urine and other biological fluids samples.

Catalog number: ARG82034

Package: 96 wells

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INTRODUCTION

Heme, or haem (spelling differences) is a substance precursive to hemoglobin, which is necessary to bind oxygen in the bloodstream. Heme is biosynthesized in both the bone marrow and the liver.

In microbiological terms, heme is coordination complex "consisting of an iron ion coordinated to a porphyrin acting as a tetradentate ligand, and to one or two axial ligands. The definition is loose, and many depictions omit the axial ligands. Among the metalloporphyrins deployed by metalloproteins as prosthetic groups, heme is one of the most widely used and defines a family of proteins known as hemoproteins. Hemes are most commonly recognized as components of hemoglobin, the red pigment in blood, but are also found in a number of other biologically important hemoproteins such as myoglobin, cytochromes, catalases, heme peroxidase, and endothelial nitric oxide synthase. [Provide by Wikipedia: Heme]

PRINCIPLE OF THE ASSAY

This Heme Assay Kit employs a convenient colorimetric method for the detection of total heme content in blood, serum, plasma, urine and other biological fluids samples. The samples or Heme standards are added to a 96 well plate. Then, a Reaction Dye and Reaction Buffer are added to convert Heme content to colored form. The absorbance of 505 nm is directly proportional to the heme concentration in the samples. The concentration of Heme samples is then determined by comparing the O.D of samples to the standard curve. The optimized formulation substantially reduces interference by substances in the raw samples and exhibits high sensitivity.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
96-Well Microplate	1 plate	4°C
Standard	1 vial (Lyophilized)	4°C
Standard Diluent	5 ml (ready to use)	4°C
Reaction Buffer	15 ml (ready to use)	4°C
Reaction Dye	1 vial (Lyophilized)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 505 nm
- Distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- All materials should be equilibrated to room temperature (RT, 20-25°C) few minutes before use.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the control and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine and other biological fluids samples: Remove particulates by centrifugation at 2-8°C and aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Serum and plasma samples can be assayed directly. Blood samples should be diluted 100-fold in distilled water.

REAGENT PREPARATION

- **Reaction Dye:** Reconstitute the Reaction Dye with **5 ml** of **distilled water**. Allow the Reaction Dye to sit for few minutes with gentle agitation to make sure the Reaction Dye is dissolved completely before use. Aliquot & store the reconstituted Reaction Dye at 4°C for up to a week is recommended.
- **Standard:** Reconstitute the Standards with **1 ml** of **Standard Diluent**. Allow the Standards to sit for few minutes with gentle agitation to make sure the Standards is dissolved completely before use. Aliquot & store the reconstituted Standards at 4°C for up to a week. Avoid repeated freeze-thaw cycles.

  Add **20 µl** of the **reconstituted standard** into **980 µl** of **Standard Diluent** to yield a working concentration of **100 µmol/L.**
- **Sample**: Samples can be used directly. (Blood sample should be diluted 1:100 with distilled water before assay) If the initial assay found samples contain Heme higher than the standard (100 µmol/L), the samples can be diluted with distilled water and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. Since the concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should assayed in duplicates is recommended. The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

1. Add **20 µL** of **standards, Blank (Distilled water) and samples** into the appropriate wells of 96-Well Microplate.
2. Add **130 µL** of **Reaction Buffer** and **50 µL** of **Reaction Dye** into each wells.
3. Mix and incubate at **room temperature for 10 mins**.
4. Read the OD with a microplate reader at **505 nm** immediately.

Summary of Heme Assay Procedure

Reagent	Sample	Standard	Blank
Sample	20 µl	-	-
Standard	-	20 µl	-
Distilled water	-	-	20 µl
Reaction Buffer	130 µl	130 µl	130 µl
Reaction Dye	50 µl	50 µl	50 µl
Mix thoroughly, incubate RT for 10 min.			
Read the OD with a microplate reader at 505 nm immediately.			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Calculation:

   Formula:

   Heme (µmol/L)

   $= C_{Standard} \times [V_{Standard} \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

   $= 100 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

   $C_{Standard}$: the standard concentration, 100 µmol/L;

   $V_{Standard}$: the volume of standard, 0.02 ml;

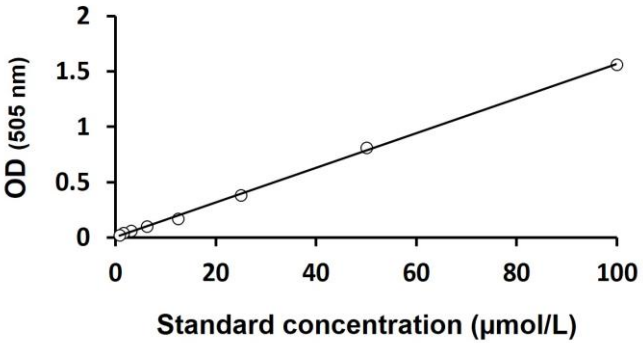
   V_{Sample}: the volume of sample, 0.02 ml.

3. Detection range:

The detection range is from 1 µmol/L to 100 µmol/L

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Heme Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results. Please note this data is for demonstration only and this kit does not need serial diluted standard.



QUALITY ASSURANCE

Sensitivity

The lowest detectable concentration of Heme is 1 µmol/L.