Heme Microplate Assay Kit

User Manual

Catalog # CAK1164

(Version 1.2A)

Detection and Quantification of Heme Content in Blood, Serum, Plasma, Urine and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Heme is one important member of the porphyrin family. Heme is 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. Heme determination is widely practiced by researchers of various blood diseases.

Heme Microplate Assay Kit is based on an improved aqueous alkaline solution method, in which the heme is converted into an uniform colored form. The intensity of color, measured at 505 nm, is directly proportional to the heme concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples and exhibits high sensitivity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	15 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Standard Diluent	5 ml x 1	4 °C
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Note:

Standard: add 1 ml Standard Diluent to dissolve, then add 20 µl standard into 980 µl Standard Diluent, the concentration will be 100 µmol/L.

Dye Reagent: add 5 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips

IV. SAMPLE PREPARATION

1. For blood, serum, plasma, urine and other biological fluids samples

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

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Reaction Buffer	130 µl	130 µl	130 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, incubate at room temperature for 10 minutes, record absorbance measured at 505 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$\text{Heme (μmol/L)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

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

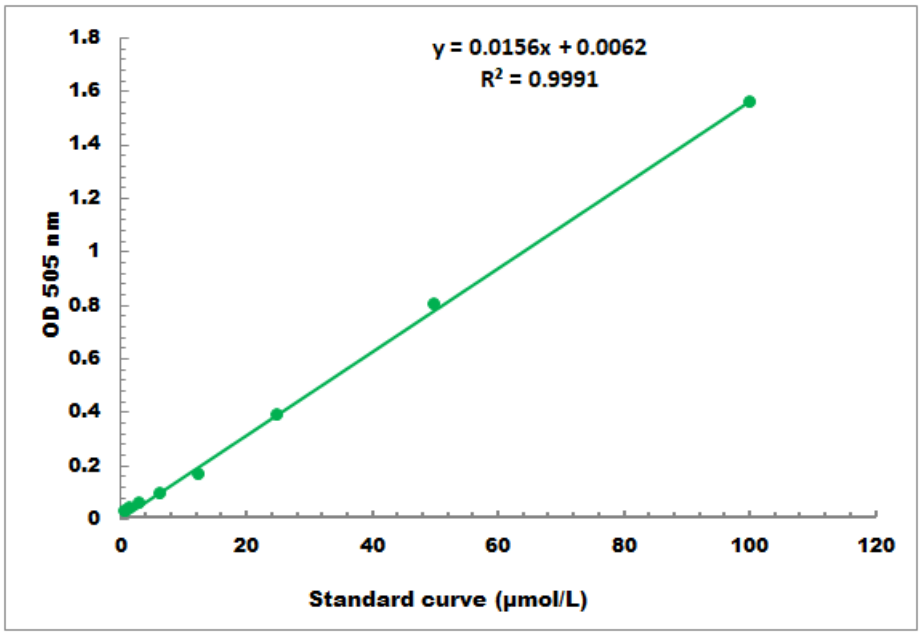
$C_{Standard}$: the standard concentration, 100 μmol/L;

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

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

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 1 μmol/L - 100 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES