Heme

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1164

(Version 1.3B)

Detection and Quantification of HemeContentin Blood, Serum, Plasma, Urineand Other biological fluidsSamples.

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.

HemeColorimetric 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	Powderx 1	4 °C, keep in dark
Standard	Powderx 2	4 °C, keep in dark
Standard Diluent	10 ml x 1	4 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolveeach vial in1 ml Standard Diluentto generate 5 mmol/Lstandardsolution.Then dilute to100 µmol/Lstandard top solutionby adding 20 µl stock solution into 980µl Standard Diluent.Perform 2-fold serial dilutions of the top standardsolution using Standard Diluentto make the standard curve.The concentration of standard curve could be100/50/25/12.5/6.25/3.12/1.56µmol/L.Standards should be used within 4 hours.

Dye Reagent: Add 5 ml distilled water to dissolve before use. Keep in dark and store at 4 °C for 12 hoursafter reconstitution.

V. SAMPLE PREPARATION

1.For blood, serum, plasma, urine and other biological fluidssamples

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

VI. 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 atroom temperature for 10minutes, record absorbance measured at 505 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/L)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

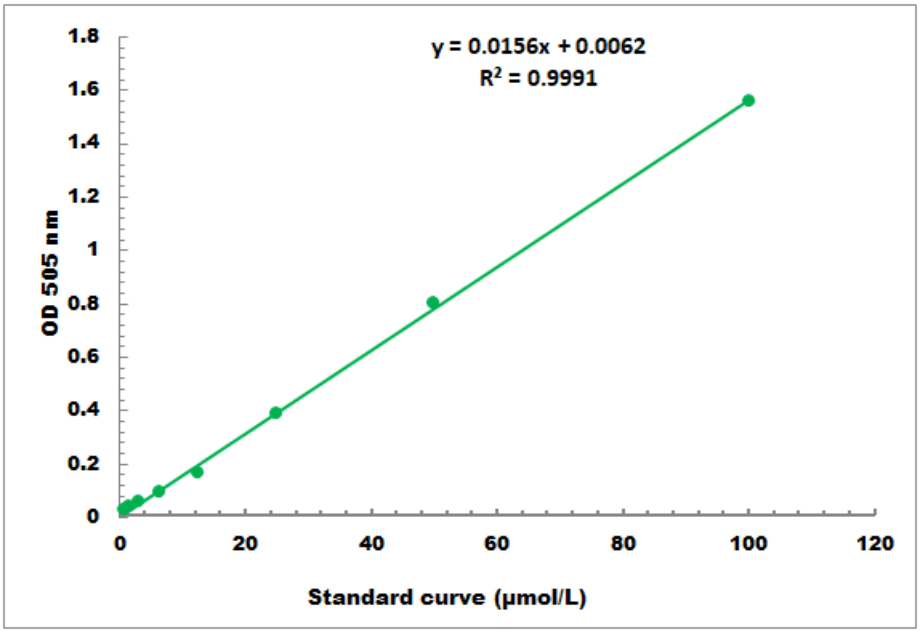
$C_{Standard}$: the standard concentration, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

VIII. TYPICAL DATA

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



Detection Range: 1µmol/L - 100 µmol/L