Hemoglobin Microplate Assay Kit

User Manual

Catalog # CAK1113

(Version 1.5F)

Detection and Quantification of Hemoglobin (Hb) Content in Serum Samples.

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

I. INTRODUCTION

Hemoglobin, also spelled haemoglobin and abbreviated Hb, is the iron-containing oxygen-transport metalloprotein in the red blood cells of the blood in vertebrates and other animals. In mammals the protein makes up about 97% of the red cell's dry content, and around 35% of the total content (including water). Hemoglobin transports oxygen from the lungs or gills to the rest of the body, such as to the muscles, where it releases its load of oxygen. Hemoglobin also has a variety of other gas-transport and effect-modulation duties, which vary from species to species, and may be quite diverse in invertebrates.

The Hemoglobin can react with O-tolidine. The products can be measured at a colorimetric readout at 435 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent A Diluent	1 ml x 1	4 °C
Dye Reagent B	4 ml x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Dye Reagent A: add 1 ml Dye Reagent A Diluent to dissolve before use; stored at -20 °C less than a month.

Standard: add 1 ml distilled water to dissolve, then add 0.1 ml standard solution into 0.9 ml distilled water, the concentration will be 100 μg/ml; stored at -80 °C less than a month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 435 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. SAMPLE PREPARATION

1. For serum sample

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Blank	Standard	Sample
Reaction Buffer	100 µl	100 µl	100 µl
Distilled water	10 µl	--	--
Standard	--	10 µl	--
Sample	--	--	10 µl
Dye Reagent A	10 µl	10 µl	10 µl
Dye Reagent B	40 µl	40 µl	40 µl
Mix, incubate at RT for 3 minutes, measured at 435 nm and record the absorbance.			

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.

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VI. CALCULATION

1. According to the serum sample

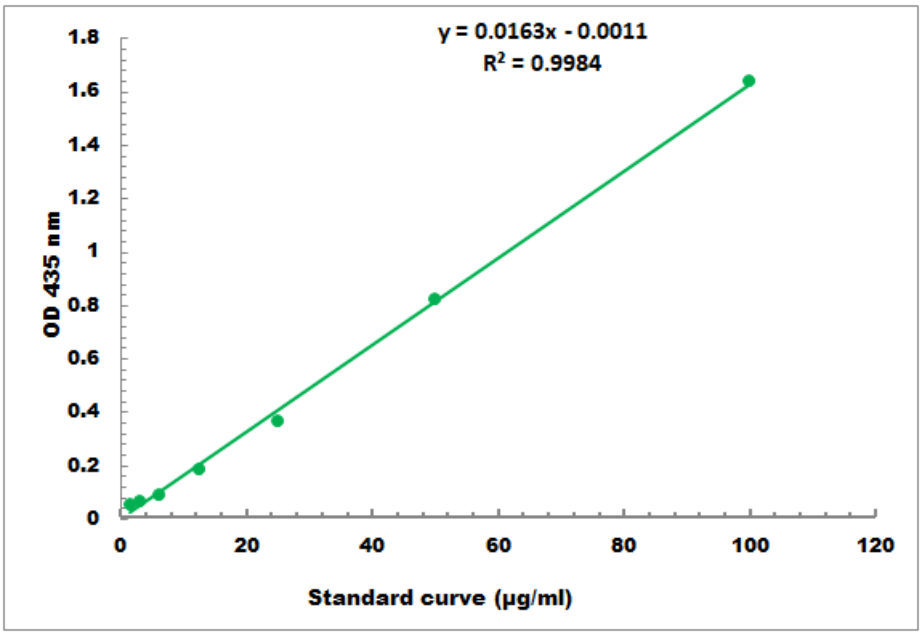
$Hb\ (\mu g/ml) = C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

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

$C_{Standard}$: the concentration of standard, 100 μg/ml.

VII. TYPICAL DATA

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



Detection Range: 1 μg/ml - 100 μg/ml

VIII. TECHNICAL SUPPORT

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

IX. NOTES