Hemoglobin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1113

(Version 1.6F)

Detection and Quantification ofHemoglobin

(Hb)ContentinSerumSamples.

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.

Hemoglobin Colorimetric Microplate Assay Kitis a sensitive assay for determining hemoglobinconcentration in various samples.Hemoglobin can react with O-tolidine.The productscan be measured at a colorimetric readout at 435 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	10 mlx 1	4 °C
Dye Reagent A	Powderx 1	4 °C
Dye Reagent ADiluent	1 mlx 1	4 °C
Dye Reagent B	4 mlx 1	4 °C
Standard	Powderx 1	4 °C
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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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate1 mg/ml of standardstock solution, store at -20 °C for 1 monthafter reconstitution.Then dilute to100 µg/mlstandard top solutionby adding0.1 ml stock solution into 0.9 mldistilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be100/50/25/12.5/6.25/3.12/1.56 µg/ml.

Dye Reagent A:Briefly centrifuge prior to opening. Add 1 ml Dye ReagentA Diluent to dissolve before use. Keep in dark and stored at -20 °C less than a month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For serum sample

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Standard	Blank	Sample*
Reaction Buffer	100 µl	100 µl	100 µl
Standard	10µl	--	--
Distilled water	--	10µl	--
Sample	--	--	10µl
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 recordthe absorbance.			

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

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

Calculate the initial concentration according to sample preparation procedure.

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

According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

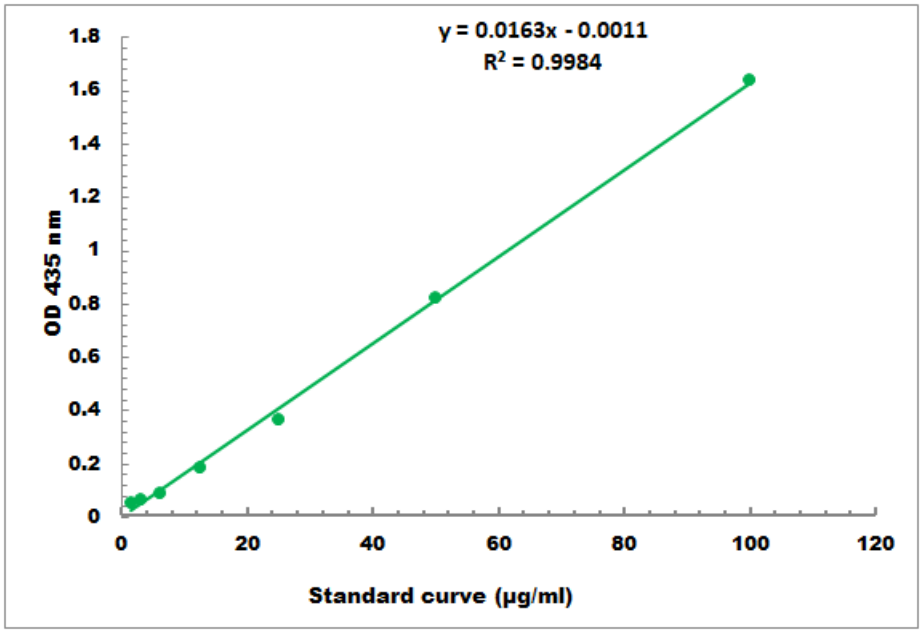
$C_{Standard}$: the standard concentration, mg/ml

$V_{Standard}$: 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: 1µg/ml -100 µg/ml