



Iron

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1106

(Version 1.7D)

Detection and Quantification of Iron(Fe^{3+}) Content in Serum, Plasma, Urine, Saliva, Tissue extracts, Cell lysate and Other biological fluids Samples.

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

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I. INTRODUCTION

Iron level in blood is a reliable diagnostic indicator of various disease states.

Increased levels of iron concentration in blood are associated with blood loss, increased destruction of red blood cells (e.g. hemorrhage) or decreased blood cell survival, acute hepatitis, certain sideroachrestic anemias, ingestion of iron-rich diets, defects in iron storage (e.g. pernicious anemia). Decreased levels of blood iron may result from insufficient iron ingestion from diets, chronic blood loss pathologies, or increased demand on iron storage as during normal pregnancy.

IronColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring iron levels in a variety of samples. The ferrium ions can react with Phenanthroline. The products can be measured at a colorimetric readout at 510 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reducing Reagent	Powder x 1	4 °C
Reaction Buffer	5 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard(500μmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 510 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. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8 $\mu\text{mol/L}$.

Reducing Reagent: Add 2.5 ml distilled water to dissolve before use. Store at 4°C for 1 week or -20 °C for 1-2 months.

Dye Reagent: Add 2.5 ml distilled water to dissolve before use. Store at 4°C for 1-2 months.

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

V. SAMPLE PREPARATION

1. For liquid sample

Liquid samples can be tested directly.

Serum or plasma samples, add 0.5 ml Assay Buffer into 0.5 ml sample, mix, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml ddH₂O for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 0.5 ml Assay Buffer mix, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.5 ml ddH₂O, then add 0.5 ml Assay Buffer mix, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Warm all the reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	50 μ l	50 μ l	50 μ l
Sample	100 μ l	--	--
Standard	--	100 μ l	--
Distilled water	--	--	100 μ l
Reducing Reagent	25 μ l	25 μ l	25 μ l
Dye Reagent	25 μ l	25 μ l	25 μ l
Mix, incubate at 37 °C for 60 mins, measured at 510 nm and record the 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. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

Calculate the initial concentration according to sample preparation procedure.

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, $\mu\text{mol/L}$

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

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

V_{Assay} : the volume of Assay Buffer, μl

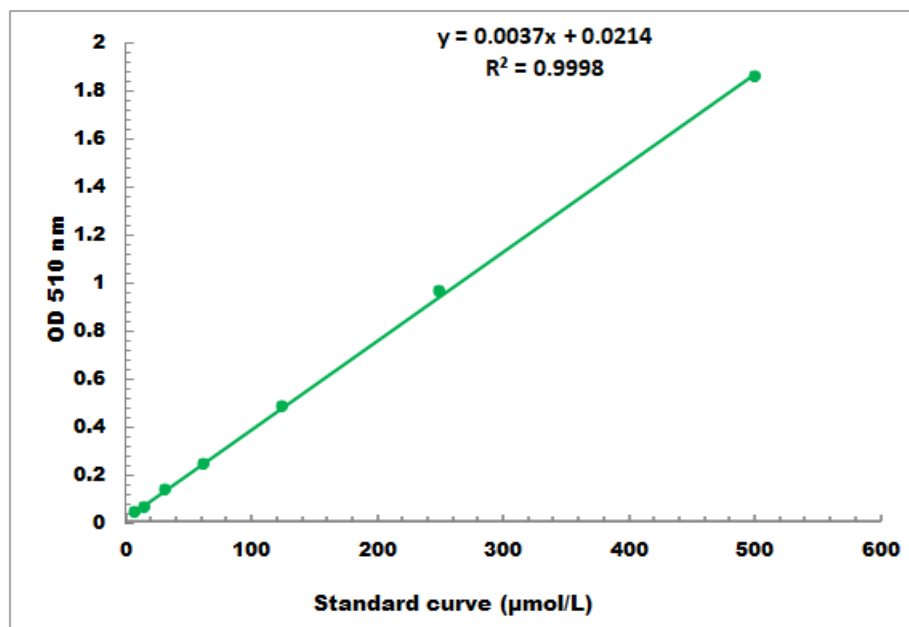
C_{Protein} : the sample protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

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



Detection Range: 5μmol/L -500μmol/L