Ferrous Iron Microplate Assay Kit

User Manual

Catalog # CAK1274

(Version 1.4B)

Detection and Quantification of Ferrous Iron (Fe^{2+}) 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

Ferrous iron (Fe^{2+}) loses an electron during conversion to the ferric (Fe^{3+}) state. This is an important component of the toxicity of ferrous iron. A similar reaction also occurs during the spontaneous oxidation of haemoglobin to methaemoglobin. It is for this reason that large quantities of SOD, catalase and other protective agents are present in the young red blood cell. Their depletion may well determine the life span of the cell. Apart from ferrous iron acting as an electron donor, it is a catalyst in the Fenton reaction.

Ferrous Iron Microplate Assay Kit provides a simple and direct procedure for measuring ferrous iron levels in a variety of samples. The ferrous iron 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
Reaction Buffer	5 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

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

Standard: add 1 ml distilled water to dissolve before use, mix; then add 10 µl into 990 µl distilled water, mix, the concentration will be 500 µmol/L.

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. 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.

V. ASSAY PROCEDURE

Warm all the reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Reaction Buffer	50 µl	50 µl	50 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, incubate at 37 °C for 60 mins, measured at 510 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.

VI. CALCULATION

1. According to the volume of sample

Fe^{2+} (μmol/ml) = ($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / V_{Sample} × 2

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

2. According to the weight of sample

Fe^{2+} (μmol/g) = ($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / (V_{Sample} × W / V_{Assay})

= 0.5 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / W

3. According to the quantity of cells or bacteria

Fe^{2+} (μmol/10^4) = ($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / (N × V_{Sample} / V_{Assay})

= 0.5 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / N

$C_{Standard}$: the concentration of Standard, 500 μmol/L = 0.5 μmol/ml;

W: the weight of sample, g;

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

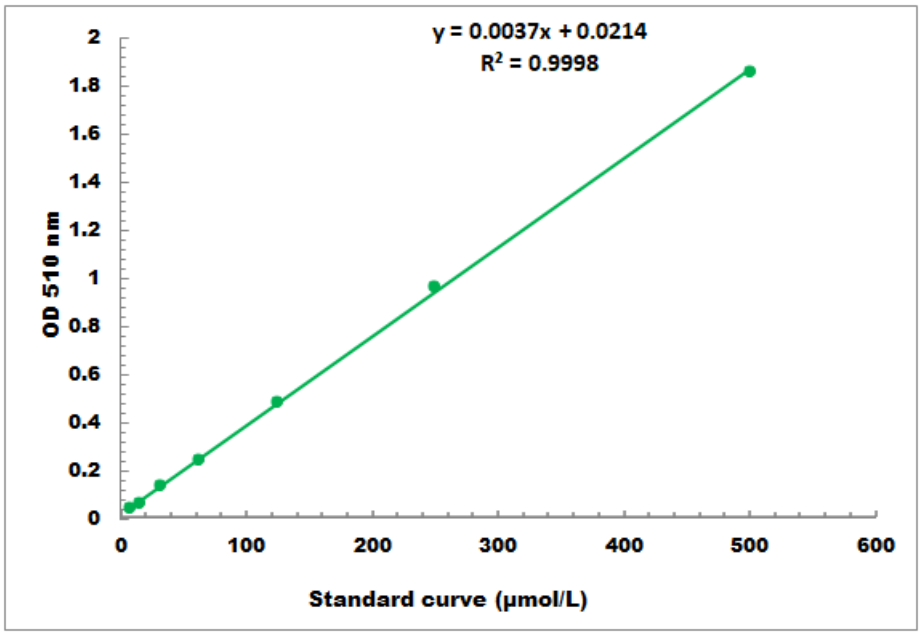
$V_{Standard}$: the volume of standard, 0.1 ml;

V_{Sample}: the volume of sample, 0.1 ml;

V_{Assay}: the volume of ddH2O + Assay Buffer, 1 ml.

VII. TYPICAL DATA

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



Detection Range: 5 μmol/L - 500 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