Iron Microplate Assay Kit

User Manual

Catalog # ASK1106

Detection and Quantification of Iron (Fe^{3+}) Content in Serum, Urine, Saliva 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.

The ferrium ions can react with Phenanthroline. The products can be measured at a colorimetric readout at 510 nm.

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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
Standard (1000 µmol/L)	1 ml x 1	4 °C
Technical Manual	1 Manual	

Note:

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

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

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 510 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. Chloroform

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IV. SAMPLE PREPARATION

1. For serum sample

Pipet 1 ml serum into a centrifuge tube, add 1 ml Assay buffer, mix, and incubate for 5 min. Add 1 ml chloroform, vortex for 10-15 sec, then centrifuge for 10 min. Add the supernatant into another centrifuge tube. The supernatant should be water clean; if not, recentrifuged.

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V. ASSAY PROCEDURE

Warm all the reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	--
Distilled water	--	--	50 µl
Reducing Reagent	50 µl	50 µl	50 µl
Reaction Buffer	50 µl	50 µl	50 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, measured at 510 nm and record the absorbance.			

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VI. CALCULATION

1. According to the serum sample

Fe^{3+} (μmol/L) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

= 1000 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

$C_{Standard}$: the concentration of Standard, 1000 μmol/L.

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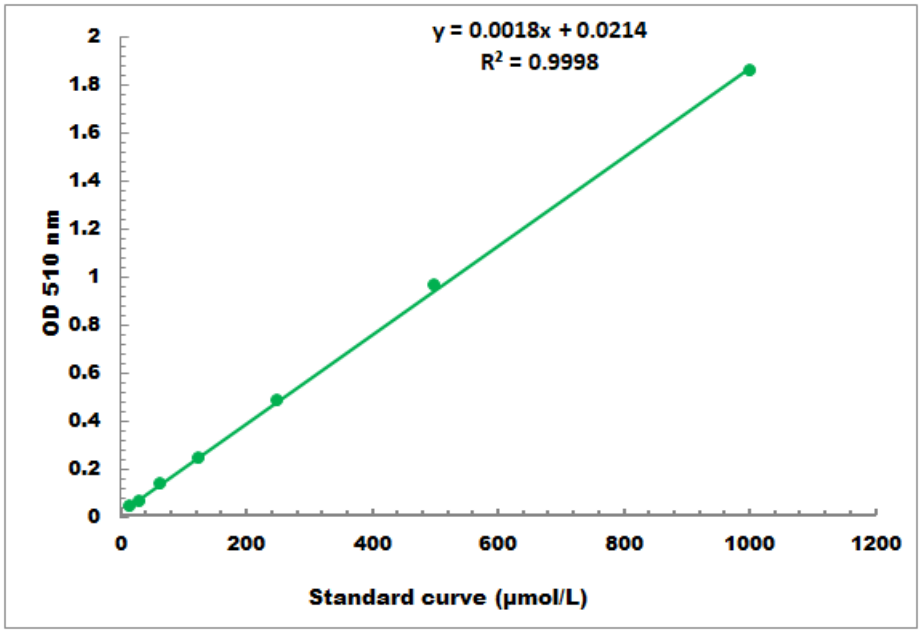
VII. TYPICAL DATA

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



Detection Range: 10 μmol/L - 1000 μmol/L

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