Ferrous Iron Assay Kit

ARG83433 Ferrous Iron Assay Kit can be used to measure Ferrous Iron in serum, plasma, urine, saliva, tissue extracts, cell lysate and other biological fluids.

Catalog number: ARG83433

Package: 96 wells

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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.

PRINCIPLE OF THE ASSAY

The Ferrous Iron Assay Kit can measure Ferrous Iron in serum, plasma, urine, saliva, tissue extracts, cell lysate and other biological fluids. This kit based on react of ferrous iron ions and Phenanthroline. The increase in absorbance at 510 nm is directly proportional to the concentration of Ferrous Iron.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml	4°C
Reaction Buffer	10 ml	4°C
Reagent Dye	1 vial (lyophilized)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 510 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- All components store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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.

Cell and Bacteria samples- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml ddH2O for 5 × 106 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.

*Note: liquid samples can detect directly.

REAGENT PREPARATION

- **Reagent Dye**: Reconstitute the **Reagent Dye** with **5 ml** of **Distilled Water.** Allow the Reagent Dye keep on bench for few minutes. Make sure the Reagent Dye is dissolved completely.
- **Standards:** Reconstitute the Standards with **1 ml** of **Distilled water**, then add **10 µl Standards buffer** into **990 µl distilled water**, the concentration will be **0.5 µmol / mL** Perform 2-fold serial dilution of the top standards to make the standard curve.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **100 µl Sample** into Sample wells.
2. Standard wells: Add **100 µl Standard** into Standard wells.
3. Add **50 µl Reaction Buffer** to each wells.
4. Add **50 µl Reagent Dye** to each wells.
5. Mix well. Incubate at **37°C** for **60 min**. Read the OD at **510nm**

Reagent	Sample	Standard	Blank
Sample	100 µl	-	-
Standard	-	100 µl	-
Distilled water	-	-	100 µl
Reaction Buffer	50 µl	50 µl	50 µl
Reagent Dye	50 µl	50 µl	50 µl
Mix well. Incubate at 37°C for 60 min. Read the OD at 510nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 0.5 μmol /ml;

V_{Sample}: the volume of reaction sample, 10 μl = 0.01 ml;

$V_{standard}$: the volume of standard, 10 μl = 0.01 ml;

V_{assay}: the volume of Assay Buffer, 1000 μl = 1 ml.

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

B. Formula:

a). According to the weight of sample

Ferrous Iron (μmol/g) =

$[(C_{Standard} \text{ X } V_{standard}) \text{ X } (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } (W \text{ X } V_{Sample} / V_{assay})]$

$=0.5 \text{ X } (OD_{Sample} - OD_{blank}) / [(OD_{Standard} - OD_{Blank}) \text{ X } W]$

b). According to the volume of sample

Ferrous Iron (μmol/ml) =

$2X \ [(C_{Standard} \text{ X } V_{standard}) \text{ X } (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } V_{Sample})]$

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

c). According to the cell or bacteria

Ferrous Iron ($\mu mol/10^4$ cell) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{assay})]$

$=0.5 \times (OD_{Sample} - OD_{blank}) / [(OD_{Standard} - OD_{Blank}) \times N]$

3. Detection range:

The detection range is from 0.5 µmol/ml – 0.005 µmol/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.