Total Iron Colorimetric Assay Kit

Catalog No: E-BC-K772-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.4 μmol/L

Detection range: 0.4-50 μmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure total iron content in serum, cells, animal and plant tissue samples.

▲Background

Iron is one of the metal elements in organism and has important physiological functions. Ferrous ion is a key element in heme and hemoglobin and plays an important role in many biochemical reactions. In recent years, with the introduction of the concept of iron death, it has been found that the absorption, transportation, storage and utilization of iron ions and their excessive accumulation in cells have a significant relationship with aging and disease.

▲Detection principle

Under the action of reductant, iron ions in samples can be reduced into ferrous ions (Fe^{2+}). The latter then bind to probe and form complexes, which has a maximum absorption peak at 593 nm. The concentration of iron can be calculated by measuring the OD value at 593 nm indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	50 mL × 2 vials	2-8℃ , 6 months, shading light
Reagent 2	Chromogenic Solution	10 mL × 2 vials	2-8℃ , 6 months, shading light
Reagent 3	10 mmol/L Iron Standard	1 mL × 2 vial	2-8℃ , 6 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Test tubes, Vortex Mixer, Centrifuge, Water bath, Microplate reader (590-600 nm, optimum wavelength: 593 nm)

Reagents:

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. Prevent the formulation of bubbles when the reagent or sample is transferred into the microplate.
2. Do not use iron appliances to prepare or transfer samples.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of 100 μmol/L iron standard:
   Mix 20 μL of reagent 3 with 1980 μL of double distilled water fully. Prepare fresh needed amount solution before use.

▲ Sample preparation

1. Serum and plasma samples:
   Mix serum sample with reagent 1 at a ratio of 1: 3 fully and preserve it on ice for detection. If the sample is turbidity, centrifuge at 5000 g for 5 min, then take the supernatant for detection.
2. Tissue sample:
   Accurately weigh the tissue, add reagent 1 at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M)
3. Cell sample:
   Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (4×10^6): reagent 1 (μL) =1: 400. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.4-50 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Mouse serum	1-2
Rat serum	1
10% Mouse liver tissue homogenate	2-3
10% Rat lung tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Rat spleen tissue homogenate	2-3
293T cells	1
10% Epipremnum aureum leaf tissue homogenate	1

Note: The diluent is reagent 1.

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	593 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note:A-H, standard wells; S1-S80, sample wells.

▲Operating steps

1. The preparation of standard curve

Dilute 100 μmol/L iron standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 30, 40, 50 μmol/L.

2. The measurement of samples

2.1. For serum and plasma

(1) Standard well: Take 200 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Take 200 μL of sample to the corresponding wells.

(2) Add 100 μL of reagent 2 to each well.

(3) Mix fully and incubate the tubes at 37℃ for 40 min.

(4) Measure the OD value of each well with microplate reader at 593 nm.

2.2 For tissue and cells

(1) Standard tube: Take 300 μL of standard solution with different concentrations to the 1.5 mL tubes.

Sample tube: Take 300 μL of sample to the 1.5 mL tubes.

(2) Add 150 μL of reagent 2.

(3) Mix fully with vortex mixer and incubate the tubes at 37℃ for 40 min.

(4) Centrifuge the tubes at 12000 g for 10 min.

(5) Take 300 μL of supernatant to the corresponding microplate wells.

(6) Measure the OD value of each well with microplate reader at 593 nm.

▲Operation table

1. For serum and plasma

	Standard well	Sample well
Standard of different concentrations (μL)	200	
Sample (μL)		200
Reagent 2 (μL)	100	100
Mix fully and incubate the tubes at 37℃ for 40 min. Measure the OD value of each well with microplate reader at 593 nm.		

2. For tissue and cells

	Standard tube	Sample tube
Standard of different concentrations (μL)	300	
Sample (μL)		300
Reagent 2 (μL)	150	150
Mix fully with vortex mixer and incubate the tubes at 37℃ for 40 min. Centrifuge the tubes at 12000 g for 10 min.Take 300 μL of supernatant to the corresponding microplate wells.Measure the OD value of each well with microplate reader at 593 nm.		

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum sample:

$$\text{Fe content (μmol/L)} = (\Delta A_{593} - b) \div a \times 4^* \times f$$

2. Tissue sample:

$$\text{Fe content (μmol/gprot)} = (\Delta A_{593} - b) \div a \times f \div C_{pr}$$

3. Cell sample:

$$\text{Fe content (nmol/10}^6\text{)} = (\Delta A_{593} - b) \div a \div (N \div V) \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{593}: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

4*: Dilution factor in the preparation step of serum, 4 times.

N: The number of cell sample. For example, the number of cells is $4*10^6$, N is 4.

V: The volume in the preparation step of cell, mL.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.4-50 μmol/L	Average intra-assay CV (%)	1.3
Sensitivity	0.4 μmol/L	Average inter-assay CV (%)	1.5
Average recovery rate (%)	99		

▲Example analysis

For rat liver tissue, take 10% rat liver tissue homogenate and dilute for 2 times, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0187 x - 0.0027, the average OD value of the sample is 0.577, the average OD value of the blank is 0.050, the concentration of protein in sample is 14.56 gprot/L, and the calculation result is:

$$\text{Fe content (μmol/gprot)} = (0.577 - 0.050 + 0.0027) \div 0.0187 \times 2 \div 14.56$$

$$= 3.89 \text{ μmol/gprot}$$