Iron Colorimetric Assay Kit

Catalog No: E-BC-K139-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.072 mg/L

Detection range: 0.072-60 mg/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 be used to measure the concentration of iron in serum and tissue samples.

▲Background

Iron is an essential element in most life forms, from bacteria to mammals. It is commonly found in metalloprotein centers, heme complexes and oxygen carrier proteins. The main form of iron stored in human body is ferritin. Iron is essential for a variety of metabolic processes, including oxygen transport, DNA synthesis and electronic transport.

▲Detection principle

Under the action of acidic solution and reductant, ferric ions can be separated from transferrin in serum, and reduced into ferrous ions (Fe^{2+}). The latter then bind to bipyridine and form pink complexes. The concentration of iron can be calculated by measuring the OD value at 520 nm indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	100 mg/L Iron Standard	2 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Chromogenic Agent A	Powder × 4 vials	2-8℃ 6 months, shading light
Reagent 3	Chromogenic Agent B	Powder × 4 vials	2-8℃ , 6 months, shading light
Reagent 4	Chromogenic Agent C	50 mL × 4 vials	2-8℃ , 6 months
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

Spectrophotometer (520 nm), Micropipettor, Vortex mixer, Incubator, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Deionized water, normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

▲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. It is recommended to use disposable consumables to avoid the contamination of iron.
2. The supernatant after centrifugation must be clarified.

Pre-assay preparation

▲Reagent preparation

1. Preparation of 2 mg/L iron standard working solution:
   Mix reagent 1 and deionized water at the volume ratio of 1:49. The prepared solution can be stored at 2-8℃ for 3 days.
2. Preparation of iron chromogenic agent:
   Dissolve 1 vial of reagent 2 and 1 vial of reagent 3 with 50 mL of reagent 4. The prepared solution can be stored at 2-8℃ for 1 month with shading light.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The sample should not contain EDTA, citrate and other metal chelators

▲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 (0.072-60 mg/L).

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

Sample type	Dilution factor
Human serum	1
Mouse serum	1
10% Mouse liver tissue homogenization	1
10% Rat kidney tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	520 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

▲Operating steps

(1) Blank tube: Add 0.5 mL of deionized water into a 5 mL EP tube.
Standard tube: Add 0.5 mL of 2 mg/L iron standard working solution into a 5 mL EP tube.
Sample tube: Add 0.5 mL of Sample into a 5 mL EP tube.

(2) Add 1.5 mL of iron chromogenic agent, mix fully with a vortex mixer, then incubate in 100℃ water bath for 5 min. (Blank tube and standard tube can be treated without 100℃ water bath.)

(3) Cool the tubes with running water, centrifuge the tubes at 2300 g for 10 min. (If the supernatant is still turbid, take the turbid supernatant into another centrifuge tube and centrifuge again.)

(4) Take 1.0 mL of supernatant. Set the spectrophotometer to zero with deionized water, and measure the OD value of each tube with spectrophotometer at 520 nm with 0.5 cm optical path quartz cuvette.

[Note]:
When taking the supernatant for colorimetry measurement, it is suggested to take the supernatant carefully with the pipette to avoid adding sediment to cuvette and affect the OD value.

▲Operation table

	Blank tube	Standard tube	Sample tube
Deionized water (mL)	0.5		
2 mg/L Iron standard working solution (mL)		0.5	
Sample (mL)			0.5
Iron chromogenic agent (mL)	1.5	1.5	1.5
Mix fully with a vortex mixer and incubate in 100℃ water bath for 5 min. Cool the tubes with running water, centrifuge the tubes at 2300 g for 10 min. Take 1.0 mL supernatant. Set the spectrophotometer to zero with deionized water, and measure the OD value of each tube with spectrophotometer at 520 nm with 0.5 cm optical quartz cuvette.			

▲Calculation

1. Serum sample:

$$\text{Iron content (mg/L)} = \frac{\Delta A_1}{\Delta A_2} \times c_1 \times f$$

or

$$\text{Iron content (μmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c_2 \times f$$

2. Tissue sample:

$$\text{Iron content (mg/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c_1 \times f \div C_{pr}$$

or

$$\text{Iron content (μmol/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c_2 \times f \div C_{pr}$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c_1: The concentration of standard, 2 mg/L

c_2: The concentration of standard, 35.8 μmol/L

2 mg/L Iron standard = 2000 μg/L ÷ Molecular weight of Iron (55.847) = 35.8 μmol/L

f: Dilution factor of sample before test.

C_{pr}: The concentration of protein in sample, gprot/L

▲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	0.072-60 mg/L	Average intra-assay CV (%)	4.6
Sensitivity	0.072 mg/L	Average inter-assay CV (%)	2.5
Average recovery rate (%)	99		

▲Example analysis

Take 0.5 mL of human serum, carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.025, the average OD value of the blank is 0.002, the average OD value of the standard is 0.040, the concentration of standard is 2 mg/L, and the calculation result is:

Iron content(mg/L)=(0.025-0.002) ÷ (0.040-0.002)×2=1.21 mg/L

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ to remove blood cells. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.