Technical Manual

Iron Colorimetric Assay Kit

- **Catalogue Code: MAES0113**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.29-50 mg/L

Sensitivity:

0.08 mg/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Iron is an essential biological element for most organisms, from bacteria to mammals. It is usually stored in metalloprotein centers, heme complexes, and oxygen carrier proteins. Most of the iron stored in the body is ferritin, but as iron is overloaded, the proportion of iron sulfur protein will increase. Iron is essential for many metabolic processes, including oxygen transport, DNA synthesis, and electron transport.

3. Intended Use

This kit can measure Iron content in serum and tissue.

4. 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.

5. Kit components & storage

Item	Specification	Storage
Iron Standard (10 mg/L)	1 mL × 2 vials	2-8˚C, 6 months
Chromogenic Agent A	2 vials Lyophilized	2-8˚C, 6 months, avoid direct sunlight
Chromogenic Agent B	2 vials Lyophilized	2-8˚C, 6 months, avoid direct sunlight
Chromogenic Agent C	20 mL × 2 vials	2-8˚C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (510-530 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. The supernatant after centrifugation must be clarified, and if there is turbidity, it must be centrifuged again.

2. During the experiment, the experimental vessel must be clean to avoid iron contamination which may affect the result of the experiment.

3. Iron chromogenic agent should be prepared in advance because the chromogenic agent A powder and chromogenic agent B powder are difficult to dissolve.

7. Reagent preparation:

Preparation of **iron chromogenic agent:** Dissolve 1 vial of chromogenic agent A and 1 vial of chromogenic agent B with 20 mL of chromogenic agent C. The prepared solution can be stored at 2-8˚C for 1 month with shading light.

8. Sample Preparation

Sample Requirements:
Metal chelating agents such as EDTA and citrate cannot be added to the sample.

1. **Serum sample:**
   Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. 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°C for a month.

2. **Tissue sample:**
   Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.29-50 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 homogenate	1
10% Rat kidney tissue homogenate	1

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

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 520 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute 10 mg/L iron standard stock solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 4, 5, 6, 8, 50 mg/L.

The measurement of samples

1. **Standard tube:** Add 75 µL of standard solution with different concentrations to the tubes.
   Sample tube: Add 75 µL of sample to the tubes.

2. Add 300 µL of iron chromogenic agent, mix fully with vortex mixer.

3. Incubate the tubes in 100°C water bath for 5 min.

4. Cool the tubes with running water, centrifuge the tubes at 3000 g for 10 min.

5. Take 200 µL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 520 nm.

Operation Table

	Standard well	Sample well
Standard solution with different concentrations (µL)	75	
Sample (µL)		75
Iron chromogenic agent (µL)	300	300
Mix fully with vortex mixer, incubate the tubes in 100℃ water bath for 5 min. Cool the tubes with running water, centrifuge the tubes at 3000 g for 10 min. Take 200 µL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 520 nm.		

11. Calculations

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:

$$\text{Fe content (mg/L)} = (\Delta A_{520} - b) \div a \times f$$

2. Tissue sample:

$$\text{Fe content (mg/gprot)} = (\Delta A_{520} - b) \div a \times f \div Cpr$$

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_{520}: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

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12. Performance Characteristics

Detection Range	0.29-50 mg/L
Sensitivity	0.08 mg/L
Average recovery rate (%)	96
Average inter-assay CV (%)	2.8
Average intra-assay CV (%)	1.2

Analysis

Take 75 µL of human serum sample, carry the assay according to the operation table. The results are as follow.

The results are as follows:

Standard curve: y = 0.01427 x + 0.0008, the average OD value of the sample is 0.078, the average OD value of the blank is 0.038, and the calculation result is:

$$\boldsymbol{Fe\ content\ (mg/L) = (0.078 - 0.038 - 0.0008) \div 0.01427 = 2.75\ mg/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching 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.

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

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