



Tissue Iron (Fe) Assay Kit

(Cat/No.: BC058 Size:50 T /48S)

1. Composition and Preparation (The kit is valid for 6 months)

Reagent No. 1: 100 mg/L iron standard stock solution: 1 mL × 1 vial, store at 4°C. When using, take 0.1 mL of the iron standard stock solution and add 4.9 mL of double-distilled water (i.e., 50-fold dilution). Prepare fresh each time.

Reagent No. 2: 1 vial of **No. 2 A powder** , 1 vial of **No. 2 B powder** , 1 bottle of **No. 2 C solution (100mL)**. Store at 4°C. When using, pour **No. 2 A powder** and **No. 2 B powder** into 100mL of **No. 2 C solution** , mix thoroughly until completely dissolved, and prepare the **iron colorimetric reagent** . Store at 4°C protected from light.

2. Required Instruments and Reagents

Visible spectrophotometer and cuvettes (or microplate reader (520nm) and 96-well plate), 95°C boiling water bath, vortex mixer, centrifuge, double-distilled water or deionized water.

3. Operating Procedures

1. Sample pretreatment:

Sample pretreatment of animal tissues : Accurately weigh the animal tissue to be tested, add 9 times the volume of physiological saline at a ratio of weight (g):volume (mL) = 1:9, mechanically homogenize under ice-water bath conditions, centrifuge at 2500 rpm for 10 minutes, and take the supernatant for testing.

Cell sample pretreatment: (Adherent cells) Scrape off cells using a cell scraper with isotonic PBS or digest with trypsin (rinse with 0.5-1 mL isotonic PBS after digestion). Transfer the cell suspension to another centrifuge tube and centrifuge at 1000 rpm for 10 minutes. Discard the supernatant and retain the cell pellet. Wash 1-2 times with isotonic buffer (0.1 mol/L, pH 7-7.4 phosphate buffer recommended) , centrifuge at 1000 rpm for 10 minutes, discard the supernatant, and retain the cell pellet (if not immediately measured, it can be stored at -20°C or -80°C and used within 3 months). Add 0.2-0.3 mL of homogenizing medium (0.1 mol/L, pH 7-7.4 PBS or physiological saline recommended) to the cell pellet. (After adding the homogenizing medium , **gently mix the cell solution to ensure homogeneity. Take a small amount for cell counting; if protein can be measured after lysis, cell counting is not necessary.**) Cells can be homogenized by sonication in an ice-water bath (power: 300W, 3-5 seconds/cycle, 30-second interval, repeated 3-5 times) or manually. If the prepared homogenate is relatively homogeneous, it can be directly analyzed without centrifugation. Alternatively, lysis buffer (Triton X-100 recommended, 1-2%, lysis for 30-40 minutes) can be used. The lysed liquid can be directly analyzed without centrifugation.

[Note]: It is recommended to have a cell count of over 1 million (the more the better the



measurement results). The homogenized liquid can be observed under a microscope to check if the cells are completely lysed.

Sample pretreatment of plant tissues : Accurately weigh the plant tissue to be tested, and add 9 times the volume of homogenizing medium (0.1 mol/L pH 7-7.4 phosphate buffer) at a ratio of weight (g): volume (mL) = 1:9. Mechanically homogenize under ice-water bath conditions, centrifuge at 3500 rpm for 10 minutes, and collect the supernatant for testing.

2、 Operation table:

	Blank tube	Standard tube	testing tube
Double-distilled water (mL)	0.5		
2 mg/L iron standard working solution (mL)		0.5	
Sample to be tested (mL)			0.5
Iron colorimetric reagent (mL)	1.5	1.5	1.5

After mixing, incubate in a boiling water bath at 95°C or above for 5 minutes. After cooling, centrifuge at 3500 rpm for 10 minutes. Take 1.0 mL of the supernatant, measure the absorbance value A of each tube using a spectrophotometer with a wavelength of 520 nm and a light path of 0.5 or 1 cm, using double-distilled water to zero the instrument (or take 200 µL of each tube and read the absorbance at 520 nm using a microplate reader).

4. Calculations and Examples

1. Calculation formula:

$$\text{Iron Content (mg/ } \mu\text{mol per g tissue)} = \frac{A_{\text{Assay}} - A_{\text{Control}}}{A_{\text{Standard}} - A_{\text{Control}}} \times C_{\text{Standard}} \div \frac{W}{V_{\text{Total Homogenate}}}$$

$$\text{Iron Content (mg/ } \mu\text{mol per } 10^4 \text{ cells)} = \frac{A_{\text{Assay}} - A_{\text{Control}}}{A_{\text{Standard}} - A_{\text{Control}}} \times C_{\text{Standard}} \div \frac{\text{Cell Count}}{V_{\text{Total Homogenate}}}$$

C_{standard} : Standard concentration, 2 mg/L (or 35.81 µmol/L);

C_{pr}: Tissue homogenate protein concentration, gprot/L (prot refers to protein).

Cell count: The total number of cells at the time of cell disruption, in tens of thousands;

W: tissue sample mass, g;

V_{_{sample total}} : The total volume of homogenizing medium added during sample pretreatment, in liters (L).

Note: The standard solution has an iron concentration of 2000 µg/L and an iron atomic weight of 55.847, so the iron content in the standard tube is 35.81 µmol/L.

Note: When calculating cell samples, the first calculation formula for tissues can also be used.

2. Calculation Example:

Example 1: 0.5 mL of 10% mouse liver tissue homogenate was taken and measured according to the procedure table. At 520 nm and a light path of 0.5 cm, the absorbance of



each tube was measured as follows: blank tube 0.002, standard tube 0.064, and test tube 0.235. The protein concentration of 10% mouse liver homogenate was also measured to be 13.1365 gprot/L. The calculations are as follows:

$$\text{Tissue Iron Content } (\mu\text{mol/gprot}) = \frac{0.237 - 0.002}{0.064 - 0.002} \times 35.81 \div 13.1365 = 10.3324 \mu\text{mol/gprot}$$

Example 2: 0.5 mL of 10% mouse kidney tissue homogenate was taken and measured according to the operation table. At 520 nm and a light path of 0.5 cm, the absorbance of each tube was measured as follows: blank tube 0.002, standard tube 0.064, and test tube 0.079. The protein concentration of the 10% mouse kidney homogenate was also measured to be 10.5776 gprot/L. The calculations are as follows:

$$\text{Tissue Iron Content } (\mu\text{mol/gprot}) = \frac{0.079 - 0.002}{0.064 - 0.002} \times 35.81 \div 10.5776 = 4.2045 \mu\text{mol/gprot}$$

Example 3: 0.5 mL of 10% mouse myocardial tissue homogenate was taken and measured according to the operation table. At 520 nm and a light path of 0.5 cm, the absorbance of each tube was measured as follows: blank tube 0.002, standard tube 0.064, and test tube 0.078. The protein concentration of the 10% mouse myocardial homogenate was also measured to be 6.6536 gprot/L. The calculations are as follows:

$$\text{Tissue Iron Content } (\mu\text{mol/gprot}) = \frac{0.078 - 0.002}{0.064 - 0.002} \times 35.81 \div 6.6536 = 6.5974 \mu\text{mol/gprot}$$

Example 4: 0.5 mL of 10% mouse lung tissue homogenate was taken and measured according to the procedure table. At 520 nm and a light path of 0.5 cm, the absorbance of each tube was measured as follows: blank tube 0.002, standard tube 0.064, and test tube 0.094. The protein concentration of the 10% mouse lung homogenate was also measured to be 7.1655 gprot/L. The calculations are as follows:

$$\text{Tissue Iron Content } (\mu\text{mol/gprot}) = \frac{0.094 - 0.002}{0.064 - 0.002} \times 35.81 \div 7.1655 = 7.4157 \mu\text{mol/gprot}$$

Example 5: 0.5 mL of 10% mouse brain tissue homogenate was taken and measured according to the operation table. At 520 nm and a light path of 0.5 cm, the absorbance of each tube was measured as follows: blank tube 0.002, standard tube 0.064, and test tube 0.040. The protein concentration of the 10% mouse brain homogenate was also measured to be 4.4358 gprot/L. The calculations are as follows:

$$\text{Tissue Iron Content } (\mu\text{mol/gprot}) = \frac{0.040 - 0.002}{0.064 - 0.002} \times 35.81 \div 4.4358 = 4.9479 \mu\text{mol/gprot}$$

Example 6: 0.5 mL of a 5% spinach leaf tissue homogenate was taken and measured according to the operating table. At 520 nm and a light path of 0.5 cm, the absorbance of each tube was measured as follows: blank tube 0.002, standard tube 0.064, and test tube 0.053. The protein concentration of the 5% spinach leaf homogenate was also measured to be 1.6378 g prot/L. The calculations are as follows:



$$\text{Tissue Iron Content } (\mu\text{mol/gprot}) = \frac{0.064 - 0.002}{0.064 - 0.002} \times 35.81 \div 1.6378 = 17.9855 \mu\text{mol/gprot}$$

5. Points to note

1. If using glassware, it must be thoroughly cleaned to avoid iron contamination. It is recommended to use disposable plastic test tubes or centrifuge tubes.
2. If the supernatant is turbid, increase the centrifugation speed or add 0.2 mL of chloroform, vortex thoroughly, centrifuge again, and then measure the color.
3. This method has good measurement results, few interfering factors, and is suitable for the measurement of various samples.
4. After the reaction is complete, 0.2 mL of the supernatant after color development can be added to a 96-well plate (be careful not to aspirate air bubbles), and the reading can be taken at 520 nm using an ELISA reader. The calculation formula remains unchanged.
5. If the iron content in the sample is too low, the amount of sample can be adjusted (increase the sample volume, keep the amount of colorimetric solution unchanged, and simultaneously increase the amount of double-distilled water in the blank tube and the amount of standard solution in the standard tube).

6. Measurement Principle

Under the action of acidic solution and reducing agent, iron in transferrin is separated from protein, and ferrous iron in tissue is reduced to ferrous iron. The latter combines with bispyridine to form a pink complex. Within a certain range, the amount of iron ions is directly proportional to the color intensity.



Appendix I: Iron Standard Curve

I. Pre-processing:

iron standard solutions of different concentrations : Take 100 mg/L iron standard stock solution and dilute it with double-distilled water to the following concentrations for testing: 0 mg/L , 0.5 mg/L , 1 mg/L , 2 mg /L , 5 mg/L , 10 mg/L , 20 mg/L , 50 mg/L .

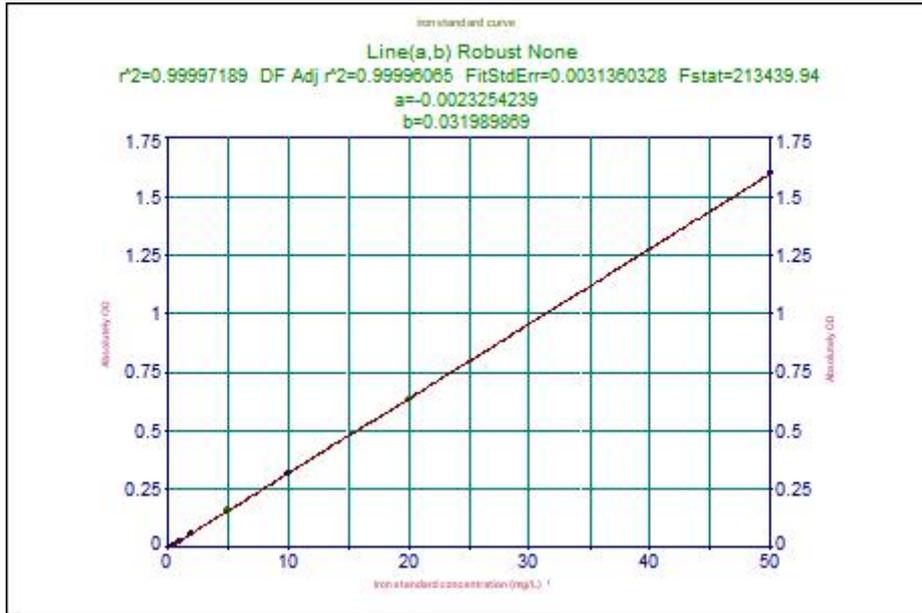
II. Operation Table:

Pipe number	1	2	3	4	5	6	7	8
iron standard solution (mg/L)	0	0.5	1.0	2.0	5.0	10	20	50
Iron standard solution sampling volume (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Iron colorimetric reagent (mL)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Mix well, incubate in a boiling water bath at 95°C or above for 5 minutes, cool with running water, use a 0.5 or 1 cm optical path (same as when measuring the sample) , a wavelength of 520 nm , zero the instrument with double-distilled water, and measure the absorbance value A of each tube with a spectrophotometer (or take a 200 µL sample from each tube and read the absorbance at 520 nm on the microplate reader) .

III. Test Results:

Pipe number	Iron standard concentration (mg/L)	Measurement of OD	Absolute OD
1	0	0.003	0.000
2	0.5	0.018	0.015
3	1	0.032	0.029
4	2	0.062	0.059
5	5	0.165	0.162
6	10	0.320	0.317
7	20	0.636	0.633
8	50	1.602	1.599



Users can skip the standard curve step and simply follow the instructions on page 1 to calculate using the formula.