Ferrous Ion Content Assay Kit (TPTZ, Micro Method)

F1515875

Storage 2-8℃. Store in the dark

Shipping Ship at low temperature. Upon receipt, store immediately at the recommended storage conditions.

Product Introduction

Iron is one of the essential elements for the human body and plays an important role in metabolism and overall health. Insufficient iron levels in the blood can lead to iron deficiency anemia. Iron is present in all cells of the human body, including various tissues, organs, and endocrine glands. The liver, spleen, and lung tissues are relatively rich in iron. The adult body contains 3–5 g of iron, most of which exists as protein complexes, with a very small portion in ionic form. Ferrous ions (Fe^{2+}) are key components of heme and hemoglobin and also play important roles in many biochemical reactions. This kit employs a colorimetric method for the quantitative determination of Fe^{2+} in animal and plant tissues, serum/plasma, or other liquids. Under acidic conditions, Fe^{2+} in the sample reacts with tripyridyltriazine (TPTZ) to form a blue complex that has a maximum absorbance at 593 nm. The concentration of Fe^{2+} is then determined by measuring the absorbance at this wavelength.

Product Components

F1515875	Components	48 T	96 T	Storage
F1515875A	Buffer Solution	15 mL	30 mL	2-8℃. Store in the dark
F1515875B	Chromogenic Solution	7 mL	14 mL	2-8℃. Store in the dark
F1515875C	Ferrous Ion Standard (40mM)	0.5 mL	1 mL	2-8℃. Store in the dark
F1515875D	Standard diluent	5 mL	10 mL	2-8℃. Store in the dark
F1515875E	Extraction Buffer	15 mL	30 mL	2-8℃. Store in the dark

Usage Protocol

1 Sample Preparation

(1) Tissue: Add 0.1 mL of extraction buffer per 0.01 g of tissue. Homogenize using a homogenizer or mortar and pestle on ice. Then incubate on ice for 15 minutes, inverting and mixing every 3 minutes to ensure complete cell lysis. Centrifuge at 10,000 × g for 10

minutes at 4°C. Collect the supernatant and keep on ice.

(2) Serum/plasma or other liquid samples: Mix 55 µL of sample with 165 µL of Buffer Solution (i.e., 4-fold dilution) and keep on ice. If the sample is turbid, centrifuge at 5,000 × g for 5 minutes at 4°C and collect the supernatant for analysis.

Note: The extraction buffer provided with this kit is not compatible with BCA protein assays. For protein concentration determination, we recommend Aladdin's Ready-to-Use Bradford Protein Assay Kit (Detergent Compatible) (B1509656). To ensure that the sample values fall within the standard curve range, it is recommended to perform a preliminary experiment with multiple dilution factors for the sample to determine the approximate concentration of ferrous ions. If the values are not within the standard curve range, please adjust the dilution factor or the amount of sample. The sample diluent is Buffer Solution; serum/plasma or other liquid samples have already been diluted 4-fold during sample processing and usually do not require further dilution.

2 Standard Curve Preparation

Dilute the 40 mmol/L (40,000 µmol/L) standard with standard diluent to obtain the following concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 µmol/L. Prepare fresh before use.

A recommended dilution scheme is shown below:

No.	Pre-dilution Conc. (µmol/L)	Std. Volume (µL)	Standard diluent Volume (µL)	Final Conc. (µmol/L)
1	40000	10	990	400
2	400	150	450	100
3	100	300	300	50
4	50	300	300	25
5	25	300	300	12.5
6	12.5	300	300	6.25
7	6.25	300	300	3.125
8	3.125	300	300	1.5625

Note: Each standard tube requires 200 µL of diluted standard for the subsequent assay (do not measure absorbance at this step).

3 Sample Measurement

(1) Pre-warm the microplate reader for at least 30 min. Set the wavelength to 593 nm.

(2) Prepare the reaction mixtures as shown below. Include a blank control (no sample).

Reagent (μL)	Blank	Standard	Sample
Sample (μL)	0	0	200
Standard (μL)	0	200	0
Buffer Solution (μL)	200	0	0
Chromogenic Solution (μL)	100	100	100

(3) Mix thoroughly and incubate at 37°C for 20 minutes. Transfer 200 μL of the supernatant to the corresponding wells of a microplate. Measure the absorbance of each well at 593 nm. Record the blank absorbance as A_{blank}, the standard as $A_{standard}$, and the sample as A_{sample}. Calculate: $\Delta A_{sample} = A_{sample} - A_{blank}$, $\Delta A_{standard} = A_{standard} - A_{blank}$.

4 Calculation of Fe^{2+} Content

4.1 Standard curve: Plot the standard concentration (x-axis) against $\Delta A_{standard}$ (y-axis) to obtain the linear equation. Substitute ΔA_{sample} into the equation to calculate the sample concentration C_{sample} (μmol/L).

4.2 Fe^{2+} concentration calculation:

4.2.1 For serum/plasma or other liquid samples: Fe^{2+} content (μmol/L) = $C_{sample} \times 4 \times f$

4.2.2 For tissue samples:

(1) Based on tissue wet weight: Fe^{2+} content (μmol/kg wet weight) = $C_{sample} \times f \div (m / V)$

(2) Based on protein concentration: Fe2+ content (nmol/mg protein) = Csample × f ÷ Cpr

Note:

f = dilution factor before measurement;

m = tissue wet weight (g);

V = volume of Extraction Buffer added (mL);

C_{pr} = protein concentration of the sample (mg/mL).

Precautions

1. Avoid contact of samples and reagents with iron materials to prevent contamination.
2. Fresh samples are recommended. Fe^{2+} is easily oxidized; prolonged storage or repeated freeze-thaw cycles may compromise accuracy.
3. If the sample reading exceeds the highest standard, dilute the sample and repeat the assay.
4. Before formal testing, a preliminary experiment using 2–3 samples with expected large differences is recommended.
5. For your safety and health, wear a lab coat and disposable gloves during operation.

Results Presentation

Typical standard curve: