Ferrous Ion Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: NA0139

Size:50T/48S

Components:

Reagent I: Liquid 80 mL×1. Store at 2-8℃.

Reagent II: Liquid 30 mL×1. Store at 2-8℃.

Standard: Powder×1, 10 mg $FeSO_4 \cdot 7H_2O$. Store at 2-8℃. Add 900 μL of distilled water and 20 μL of concentrated sulfuric acid before use and shake to dissolve. The Fe^{2+} standard solution of 40 mmol/L could be stored at 2-8℃ for two weeks.

Product Description:

Iron is one of the essential trace elements in human body. Fe^{2+} is the main component of hemoglobin, myoglobin, cytochrome and other enzyme systems, which could assist in the transport of oxygen and promote fat oxidation. Iron deficiency can easily cause anemia, metabolic disorders, and affect the immune function of the body.

Fe^{2+} could react with Tripyridyltriazine to form a kind of blue compound under acid condition, which has an absorption peak at 593 nm. Changes of the absorbance at 593nm could be measured to reflect ferrous ion content.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, balance, low temperature table centrifuge, constant temperature incubator/water bath, 1mL glass cuvette, pipette, mortar/homogenizer/cell ultrasonic crusher, ice, distilled water and **concentrated sulfuric acid**.

Procedure:

I. Sample preparation:

1. **Tissue:** according to tissue weight (g): Reagent I volume (mL) is 1:5-10. (It is recommended that add 1 mL of Reagent I to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 10000 g for 10 minutes at 4℃. Take the supernatant for test.
2. **Bacteria/cells:** according to the number of bacteria/cells (10^4): the volume of Reagent I (mL) is 500~1000:1. It is suggested that add 1 mL of Reagent I to 500 million of cells. Breaking bacteria/cells by ultrasonic wave in ice bath (power 200W, ultrasonic 3s, interval 7s, total time 5 min). Centrifuge at 10000 g 4℃ for 10 minutes. Take the supernatant on ice for test.
3. **Serum (plasma) or other liquid samples:** detect directly. Centrifuge before detecting if there are precipitation in the liquid.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 593 nm, set zero with distilled water.

2. Standard working solution: Prepare 400 μmol/L standard solution with 10 μL of 40 mmol/L standard solution and 990 μL of distilled water. Dilute 400 μmol/L standard solution with Reagent I to 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 μmol/L for standby.
3. Add reagents with the following list:

Reagent (μL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	800	-	-
Standard solution	-	800	-
Reagent I	-	-	800
Reagent II	400	400	400
Mix thoroughly. React at 37°C for 10 minutes.			
Trichloromethane	200	-	-
Mix thoroughly for 5 minutes and centrifuge at 12000 g for 10 minutes at room temperature. Take 800μL upper inorganic phase solution in 1mL glass cuvett. Measure absorbance at 593 nm, recorded as A_T. $\Delta A_T = A_T - A_B$.		Measure absorbance at 593 nm, recorded as A_B, and A_S. $\Delta A_S = A_S - A_B$. Blank tube and standard curve only need to test once or twice.	

III. Ferrous Ion Content Calculations

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔA_T into the equation to get x (μmol/L).

2. Calculation

1) Serum (plasma) or other liquid samples: Ferrous ion content (μmol/L) =x
2) Protein concentration: Ferrous ion content (μmol/mg prot) $=x\times10^{-3}\times V_E\div(Cpr\times V_E)=0.001x\div Cpr$
3) Sample weight: Ferrous ion content (μmol/g weight) $= x\times10^{-3}\times V_E\div W=0.001x\div W$
4) Bacteria/cells number: Ferrous ion content (μmol/10^6 cell) $= x\times10^{-3}\times V_E\div N=0.001x\div N$

Cpr: Supernatant sample protein concentration, mg/mL;

V_E: Added Reagent I volume, 1 mL;

W: Sample weight, g;

N: Total number of bacteria or cells, per 10^6;

10^{-3}: Unit conversion factor, 1 μmol/L=10^{-3} μmol/mL.

Note:

1. It is better to prepare standard solution before using because standard solution diluted with Reagent I easily fail.
2. If A_T is close to A_B or ΔA_T is too low, it is recommended to increase sample supernatant size before determination. If $A_T > 1$, it is recommended to dilute sample supernatant with Reagent I before determination. And modify the calculation formula.

3. It is suggested to take upper inorganic phase solution carefully to avoid taking lower trichloromethane solution.

Experimental example:

1. Take 0.1026g mouse liver, add 1 mL of Reagent I, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T = A_T - A_B$ =0.315-0.01=0.305. Bring the result into the standard curve y=0.0104x-0.008 and calculate x=30.096. The result is calculated according to sample weight:
   Ferrous ion content (μmol/g weight) =0.001x÷W =0.293 μmol/g weight.
2. Take 800μL of calf serum, operate according to the determination steps, calculate $\Delta A_T = A_T - A_B$ =0.400-0.01=0.390. Bring the result into the standard curve y=0.0104x-0.008 and calculate x=38.269. The result is calculated according to liquid volume:
   Ferrous ion content (μmol/L) =x=38.269 μmol/L.

References：

[1] Dong C, Yang M, Wang W. Study on spectrophotometric determination of Fe(II) and Fe(III) with 2, 4, 6- tri(2′-pyridyl)-1, 3, 5-triazine. [J]. Chinese Journal of Analysis Laboratory, 2004, (01):76-78.

[2] Huang Y, Ma H, Xu J, et al. Development and Validation of Reference Methods for Determination of Serum iron. [J]. Chinese Journal of Laboratory Diagnosis, 2011, 15(03):453-457.

[3] Wang H, Liu B, Ding Z, et al. Ferene method flow injection analysis as optimized in situ analysis of dissolved iron in marine waters. [J]. Marine Sciences, 2016, 40(05):82-87.

Related Products：

NA0736/NA0494 Serum Ferri Ion Content Assay Kit
NA0666/NA0424 Blood Zinc Content Assay Kit
NA0661/NA0420 Serum Total Iron Binding Capacity(TIBC) Assay Kit
NA0302/NA0301 Tissue Iron Content Assay Kit