# **Tissue Iron Content Assay Kit**

Note: Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

Cat Number: BC4350

**Size:** 50T/48S

### **Components:**

Extract solution: Liquid 55 mL×1. Storage at 4°C.

Reagent I: Powder×2. Storage at 4°C. Add 7.5 mL of distilled water before use. Prepare the reagent when it will be used. When the reagent turns black, it cannot be used;

Reagent II: Powder $\times$ 2. Storage at 4°C. Add 375  $\mu$ L of glacial acetic acid and 12 mL of distilled water before use. Unused reagent can be stored for one week at 4 °C.

Standard Solution: Liquid 3 mL×1, 1 µmol/mL Fe<sup>3+</sup> standard solution. Storage at 4°C. Add distilled water dilute 8 times to form a standard solution of 0.125 µmol/mL before use. Prepare when the solution will be used.

# **Product Description:**

Iron is one of the essential trace elements in human body, which is the main component of hemoglobin, myoglobin, cytochrome and other enzyme systems. Iron can 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<sup>3+</sup> is reduced by sodium sulfite to Fe<sup>2+</sup>, which reacts with 2,2-dipyridine-bipyridine, have an absorption peak at 520 nm. According measure absorbance at 520 nm can reflect tissue iron concentration.

## Reagents and Equipment Required but Not Provided.

Spectrophotometer, centrifuge, chloroform, adjusted transferpettor, mortar/homogenizer, 1 mL glass cuvette, chloroform, ice and distilled water.

# **Procedure:**

Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenize on ice bath. Centrifuge at 4000×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant for test.

#### **Detection:**

- 1. Preheat the spectrophotometer for 30 minutes, adjust wavelength to 520 nm, set zero with distilled water.
- 2. Add reagents with the following list:

Reagent Name (μL)	Blank tube (A <sub>B</sub> )	Test tube (A <sub>T</sub> )	Standard tube (A <sub>S</sub> )
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Distilled water	400	-	-
Standard solution (0.125 µmol/mL)	-	-	400
Sample	-	400	-
Reagent I	200	200	200
Reagent II	400	400	400

Mix thoroughly, incubate in boiling water bath for 5 minutes, cooling liquid. Add 200 $\mu$ L of chloroform. Shake well and centrifuge at 10000 rpm for 10 minutes at room temperature. Take 700 $\mu$ L of supernatant to 1 mL glass cuvette. Measure absorbance at 520 nm. Recorded as  $A_B$ ,  $A_T$ ,  $A_S$ . Calculate  $\Delta A_{T}=A_T-A_B$ ,  $\Delta A_S=A_S-A_B$ .

## III. Calculation

# 1) Tissue weight

Tissue iron ( $\mu g/g$ ) =Cs× $\Delta A_T \div \Delta A_S \times Ve \times 55.845 \div W =6.98 \times \Delta A_T \div \Delta A_S \div W$ 

# 2) Tissue protein concentration

Tissue iron ( $\mu$ g/mg prot) =Cs× $\Delta$ A<sub>T</sub>÷ $\Delta$ A<sub>S</sub>×Ve×55.845÷(Cpr×Ve) =6.98× $\Delta$ A<sub>T</sub>÷ $\Delta$ A<sub>S</sub>÷Cpr

Cs: Fe<sup>3+</sup> standard solution, 0.125 µmol/mL;

55.845: Relative molecular mass of Fe, 55.845 μg/μmol;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

W: Sample weight, g.

#### Note:

- 1. When  $\Delta A > 1$ , please dilute the sample to appropriate concentration with distilled water, multiply dilute times in the formula.
- 2. Reagent I cannot be used if it becomes black after dissolution. Reagent II is toxic, take self-protection measures when using.

## **Related products:**

BC2860/BC2865 Serum Total Iron Binding Capacity(TIBC) Assay Kit

BC2830/BC2835 Water Chromium(VI) Content Assay Kit

BC2840/BC2845 Phosphate Content Assay Kit

BC2850/BC2855 Total Phosphorus Content Assay Kit

#### **Technical Specifications:**

Minimum Detection Limit: 0.00009449 µmol/mL

Linear Range: 0.0039-0.25 µmol/mL