

# **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 2-8°C.

**Reagent I:** Powder×2. Storage at 2-8°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, the unused reagent can be stored at 2-8°C for 1 weeks.

**Reagent II:** Powder×2. Storage at 2-8°C. Add 375  $\mu$ L of glacial acetic acid and 12 mL of distilled water before use. Unused reagent can be stored for 1 week at 2-8°C.

**Standard Solution:** Liquid 3 mL×1, 1  $\mu$ mol/mL Fe<sup>3+</sup> standard solution. Storage at 2-8°C. Add distilled water dilute 8 times to form a standard solution of 0.125  $\mu$ mol/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.

 $Fe^{3+}$  is reduced by sodium sulfite to  $Fe^{2+}$ , 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, acetic acid, 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  $4000 \times g$  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:

	0		
Reagent Name (µL)	Blank tube (A <sub>B</sub> )	Test tube (A <sub>T</sub> )	Standard tube (As)

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Distilled water	400	-	-
Standard solution (0.125 µmol/mL)	-	7 JFE -	400
Sample	-	400	ALD NOES
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 800 $\mu$ L of supernatant to 1 mL glass cuvette. Measure absorbance at 520 nm. Recorded as A<sub>B</sub>, A<sub>T</sub>, A<sub>S</sub>. Calculate  $\Delta A_T = A_T - A_B$ ,  $\Delta A_S = A_S - A_B$ . The standard tube and blank tube only need to be measured 1-2 times.

#### **III.** Calculation

## 1) Tissue weight

Tissue iron ( $\mu g/g$ ) =Cs× $\Delta A_T$ + $\Delta A_S$ ×Ve×55.845+W =6.98× $\Delta A_T$ + $\Delta A_S$ +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. If  $\Delta A$  is too small, it can be determined by increasing the reaction time (1hour or 2 hours) or increasing the volume of sample.

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



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