

## Tissue Iron Content Assay Kit

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

**Operation Equipment:** Spectrophotometer/ Microplate Reader

**Cat Number:** AK0077

**Size:** 100T/96S

### Components:

Extract solution: Liquid 110 mL×1. Storage at 4°C .

Reagent I: Powder×2. Storage at 4°C . Add 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×2. Storage at 4°C . Add 235μL of glacial acetic acid and 7.5 mL of distilled water before use. Unused reagent can be stored for one week at 4 °C .

Standard Solution: Liquid 3 mL×1, 1μmol/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.

Microplate reader or spectrophotometer, centrifuge, chloroform, adjusted transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, chloroform, ice and distilled water.

### Procedure:

Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized 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/microplate reader 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> )
Distilled water	120	-	-

Standard solution (0. 125 μmol/mL)	-	-	120
Sample	-	120	-
Reagent I	60	60	60
Reagent II	120	120	120

Mix thoroughly, incubate in boiling water bath for 5 minutes, cooling liquid. Add 60μL of chloroform. Shake well and centrifuge at 10000 rpm for 10 minutes at room temperature. Take 200μL of supernatant to micro glass cuvette or 96 well plate. Measure absorbance at 520 nm. Recorded as  $A_B$ ,  $A_T$ ,  $A_S$ ,  $\Delta A_T = A_T - A_B$ ,  $\Delta A_S = A_S - A_B$ .

### III. Calculation

#### 1) Tissue weight

Tissue iron ( μg/g weight) =  $C_s \times \Delta A_T \div \Delta A_S \times V_e \times 55.845 \div W = 6.98 \times \Delta A_T \div \Delta A_S \div W$

#### 2) Tissue protein concentration

Tissue iron (μg/mg prot) =  $C_s \times \Delta A_T \div \Delta A_S \times V_e \times 55.845 \div (C_{pr} \times V_e) = 6.98 \times \Delta A_T \div \Delta A_S \div C_{pr}$

$C_s$ : Fe<sup>3+</sup> standard solution, 0. 125μmol/mL;

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

$V_e$ : Extract solution volume, 1 mL;

$C_{pr}$ : Supernatant sample protein concentration (mg/mL);

$W$ : Sample weight, g.

#### Note:

1. When  $\Delta A > 0.6$ , 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 :

AK0374/AK0373	Serum Total Iron Binding Capacity(TIBC) Assay Kit
AK0175/AK0174	Water Chromium(VI) Content Assay Kit
AK0378/AK0377	Phosphate Content Assay Kit
AK0376/AK0375	Total Phosphorus Content Assay Kit

#### Technical Specifications :

Minimum Detection Limit: 0.00206 μmol/mL

Linear Range : 0.0039-0.25 μmol/mL