Tissue Iron Content Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat Number: BC4355

Size: 100T/96S

Components:

Extract solution: Liquid 110 mL×1. Storage at 2-8℃.

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

Reagent II: Powder×2. Storage at 2-8℃. Add 235μL of glacial acetic acid and 7.5 mL of distilled water before use. Unused reagent can be stored for 1 week at 2-8℃.

Standard Solution: Liquid 3 mL×1, 1μmol/mL Fe^{3+} standard solution. Storage at 2-8℃. Add distilled water dilute 8 times to form a standard solution of 0.125μmol/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.

Microplate reader or spectrophotometer, centrifuge, adjusted transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, chloroform（≥98%，AR）, acetic acid（≥98%，AR）, 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℃ 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_B)	Test tube (A_T)	Standard tube (A_S)

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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 (wrap the sealing film to prevent explosion cover), 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. $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation

1) Tissue weight

Tissue iron (μg/g weight) $=Cs\times\Delta A_T\div\Delta A_S\times Ve\times55.845\div W=6.98\times\Delta A_T\div\Delta A_S\div W$

2) Tissue protein concentration

Tissue iron (μg/mg prot) $=Cs\times\Delta A_T\div\Delta A_S\times Ve\times55.845\div(Cpr\times Ve)=6.98\times\Delta A_T\div\Delta A_S\div Cpr$

Cs: Fe^{3+} standard solution, 0.125μmol/mL;

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

Ve: Extract solution volume, 1 mL;

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

W: Sample weight, g.

Note:

1. When ΔA>0.6, please dilute the sample to appropriate concentration with distilled water, multiply dilute times in the formula. If ΔA 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: 0.00206 μmol/mL

Linear Range: 0.0039-0.25 μmol/mL

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