Instruction Manual

Iron Assay Kit (Microanalysis)

Description

Iron is a metallic element that plays a crucial role in numerous biological processes, including iron transport and redox reactions. As a transition element, iron can exist in multiple oxidation states, with the most common being ferrous iron (Fe^{2+}, iron II) and ferric iron (Fe^{3+}, iron III). Iron-containing proteins participate in a wide range of biochemical reactions, often utilizing transient changes in iron oxidation states to drive chemical processes.

Serum iron refers to the iron bound to transferrin in the bloodstream. This parameter is commonly used to differentiate iron-deficiency anemia from non–iron-deficiency anemia.

Detection Principle

Sodium sulfite reduces serum Fe^{3+} to Fe^{2+}, which subsequently reacts with 2,2'-bipyridine to form a colored complex with an absorption peak at 520 nm. The serum iron content can therefore be determined by measuring the absorbance at this wavelength.

Packing

Taking 100T/96S packing for example:

Components	Packing	Storage
CB0201M-A	1 vial (powder) x 2	Store at 4°C. Prepare freshly before use and dissolve completely in 7.5 mL of distilled water.
CB0201M-B	1 vial (powder) x 2	Store at 4°C. Prepare fresh before use by adding 235 μL of glacial acetic acid, then add 7.5 mL of distilled water and dissolve thoroughly.
CB0201M-Standard	1 vial (solvent) x 1	100 μmol/L Fe^{3+} standard solution, stored at -20°C.

* Before formal testing, perform a preliminary test using 2–3 samples with relatively large expected differences.

Instructions

I. Self-Prepared Equipment

Visible spectrophotometer/microplate reader, micro glass cuvettes/96-well plates, refrigerated centrifuge, water bath, adjustable pipettes, glacial acetic acid, chloroform, and distilled water.

II. Sample Handling

Direct detection in serum (plasma)

III. Assay Procedure

1. Preheat the spectrophotometer for at least 30 minutes, set the wavelength to 520 nm, and use distilled water to zero the instrument.
2. Standard solution thawing: Remove the standard solution in advance and allow it to thaw completely at room temperature, then mix thoroughly.
3. Sample measurement (add the following reagents sequentially into EP tubes):

	Blank Tube (μL)	Standard Tube (μL)	Sample Tube (μL)
Distilled Water	125		
CB0201M-A	125	125	125
CB0201M-B	125	125	125
CB0201M-Standard Solution		125	
Serum			125
Mix thoroughly, cap tightly, incubate in a boiling water bath for 5 minutes, then cool with running tap water.			
Chloroform (self-prepared)	62	62	62

Mix thoroughly by vortexing. Centrifuge at 10,000 rpm for 10 min at room temperature. Carefully transfer 210 μL of the supernatant into a micro glass cuvette or a 96-well plate, and measure the absorbance at 520 nm. Record the readings as A_blank, A_standard, and A_sample.
The blank tube and standard tube only need to be measured 1–2 times, respectively.

IV. Calculation of Iron Concentration

Serum Iron Content (μmol/L) = C×(A1-A2)÷(A3-A2) = 100 ×(A1-A2)÷(A3-A2)
A1: A_sample
A2: A_blank
A3: A_standard
C: 100 μmol/L Fe3+ standard solution

Precautions

1. The serum iron content is low, so special attention should be paid to the containers used (e.g., EP tubes) to avoid iron contamination.
2. The solutions CB0201M-A and CB0201M-B are unstable and should be freshly prepared before use. Newly prepared reagents should be used up on the same day.
3. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
4. Please wear a lab coat and disposable gloves.