Serum Iron Concentration Assay Kit(Micromethod)

Catalogue No.: K201

Size: 100T(96 samples)

Kit component:

Item	Quantity	Instructions	**Storage**
Standard	Liquid 2mL×1 bottle	1000 μmol/L Fe^{3+} standard, diluted 8 times to 125μmol/L standard before use.	2-8℃ for 3 months
Reagent 1	Powder×2 bottles	Add 7.5mL distilled water to dissolve fully before use.	2-8℃ for 3 months
Reagent 2	Powder×2 bottles	Add 235μL glacial acetic acid and 7.5 mL distilled water to dissolve fully before use.	2-8℃ for 3 months

Principle of the Assay:

Serum iron refers to the iron bound by blood transferrin, which is often used to distinguish iron deficiency from non-iron deficiency anemia. Serum Fe^{3+} is reduced by sodium sulfite to form Fe^{2+}, and Fe^{2+} is further colored with 2, 2 '-bipyridine. There is an absorption peak at 520nm, and the serum iron content could be calculated by measuring the light absorption value at this wavelength.

Detection method:

Micromethod

Materials Not Supplied:

Centrifuge, Adjustable pipette, Visible spectrophotometer/Microplate reader, Microglass cuvettes/96-well plate, Glacial acetic acid, Chloroform, Distilled water.

Assay Procedure (For reference):

1. Sample preparation

Serum (plasma): Directly detect.

2. Add each reagent in turn according to the operation table

Before the formal test, 2-3 samples with significant differences should be selected for pre-test.

(1) Preheat visible spectrophotometer or microplate reader for more than 30 min, adjust the wavelength to 520 nm and the distilled water to zero.

(2) Add each reagent in turn according to the operation table

Reagent（μL）	Sample tube	Standard tube	Blank tube
Serum /plasma	125	-	-
125μmol/mL standard	-	125	-
Distilled water	-	-	125
Reagent 1	125	125	125
Reagent 2	125	125	125
Mix well, cover tightly, place in boiling water bath for 5min, cool with tap water. Add 62 μL chloroform（self-prepared）and mix thoroughly. At room temperature 10000rpm, centrifuge for 10min, carefully absorb 210 μL of the upper liquid, add it into the microglass cuvettes/96-well plate, and immediately measure the absorbance at 520 nm, recorded as A blank, A sample, and A standard.			

3. Calculation

The content of serum iron(μmol /L)=[C standard×(A sample- A blank) ÷ (A standard- A blank)]

=125×(A sample- A blank) ÷(A standard- A blank)

C standard: 125 μmol/L Fe^{3+} standard

Notes:

1. The content of serum iron is low, and the vessels used (EP tubes) need to be paid attention to avoid being contaminated by iron.
2. If the absorbance value of the sample is greater than 0.5, it is recommended to dilute the sample with distilled water before measurement.
3. Sensitivity: 0.99 μmol/L
4. Range: 3.9-250 μmol/L