GENLISA® Blood Serum Iron Assay

(Colorimetric Method)

Cat No: KBCA1226

Pack Size: 50Tests

1. INTRODUCTION

This kit can be used for laboratory research only.
Assay principle: Add excess iron in blood serum to make all transferrin combined with iron, then add adsorbent to adsorb redundant iron. Use blood serum iron assay method to measure iron content, this value is considered as total iron-binding capacity (TIBC), TIBC – Blood serum iron content = unsaturated iron-binding capacity (UIBC).

2. REAGENT COMPOSITION & PREPARATION

100mg/l (1791 mol/l) iron standard stock solution: 6ml×1, can be stored at 4ºC for 3 months.
10mg/l (179.1 mol/l) iron standard stock solution preparation: Dilute stock solution 10 times (for example, take 5ml stock solution and add distilled until volume reaches to 50ml.
1mg/l (17.91 mol/l) iron standard working solution preparation: Dilute iron standard working solution 10 times (for example, take 1ml 10mg/l (179.1 mol/l) iron standard working solution and add distilled water until volume reaches to 10ml).
Iron adsorbent: 50mg×50 vials, can be stored at 4℃for 6 months.
Iron chromogenic agent: Powder A×1 vial, Powder B×1 vial, Liquid B×1 bottle, can be stored at 4℃for 6 months.
When use, pour Powder A and Powder B in Liquid C, mix sufficiently to dissolve completely, iron chromogenic agent is prepared. Can be stored at 4 ºC ~8℃away from light.

3. REQUIRED EQUIPMENTS & REAGENTS

- A spectrophotometer capable of measuring absorbance at 520nm, cuvettes of 0.5cm light path
- Boiling water bath
- Micropipets and tips
- Vortex mixer
- Desk centrifuge
- A source of pure water (preferably double distilled water and double distilled water)

4. OPERATION PROCEDURE

Sample pretreatment: Take 1ml blood serum (or plasma), add 1ml 179.1 mol/l iron standard working solution, mix sufficiently, place for 10 minutes, add 50mg iron adsorbent, mix sufficiently, place at room temperature for 5 minutes, mix sufficiently again, mix 4 times in total, centrifugate at 3000~3500rpm for 10 minutes (use desk centrifuge), take 1ml supernatant, operate according to table below.

Operation table

	Blank tube	Standard tube	Sample tube
Distilled water (ml)	1		
1mg/l (17.91 mol/l) iron standard working solution (ml)		1	
Supernatant (ml)			1
Iron chromogenic agent (ml)	2	2	2

Mix sufficiently, place in boiling water bath for 5 minutes (blank tube and standard tube can skip this step), cool to room temperature, centrifugate at 3500rpm for 10 minutes, take 1.5ml supernatant each tube, transfer in cuvettes of 0.5cm light path, measure OD values of all tube at 520nm (adjust zero by double distilled water).

5. CALCULATION

(1) Formula:

$$\text{TIBC(mg/L)}=\frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}}\times\frac{\text{Standard concentration}}{(1\text{mg/L})}\times\text{Sample dilution times before assay (2)}$$

$$\text{TIBC(μmol/L)}=\frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}}\times\frac{\text{Standard concentration}}{(17.91\,\mu\text{mol/L})}\times\text{Sample dilution times before assay (2)}$$

UIBC(μmol/L)=TIBC-Blood serum iron content

$$\text{Iron saturation degree}=\frac{\text{Blood serum iron content}}{\text{TIBC}}\times 100\%$$

(2) Example:

Take blood serum to measure TIBC, in results, ODBlank is 0.000, ODStandard is 0.028, ODSample is 0.056, blood serum iron content is 26.50 mol/l, calculate as follows:

$$\text{TIBC(μmol/L)}=\frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}}\times\frac{\text{Standard concentration}}{(17.91\,\mu\text{mol/L})}\times\text{Sample dilution times before assay (2)}$$

$$=\frac{0.056-0.000}{0.028-0.000}\times 17.91\times 2$$

$$=71.64\ \mu mol/L$$

UIBC(μmol/L)=TIBC-Blood serum iron content

=71.64-26.50=45.14μmol/L

$$\text{Iron saturation degree}=\frac{\text{Blood serum iron content}}{\text{TIBC}}\times 100\%$$

=26.50/71.64×100%=36.99%

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