ELK Biotechnology

For research use only.

# Blood Serum Iron Assay Kit

(Item No.: BC034 Colorimetric Method)50T/48S

## 1. INTRODUCTION

This kit can be used for laboratory research only.

Assay principle:

Under effects of acidic solution and reductant, iron in transferrin is separated from protein, Fe3+ in serum is reduced to Fe2+, Fe2+ combines with dipyridine to produce pink complex. In a certain range, iron content appears direct proportion with absorbance.

## 2. REAGENT COMPOSITION & PREPARATION

100mg/L iron standard stock solution: Solution 1ml×bottle, can be stored at 4℃ for 3 months.

2mg/L iron standard working solution preparation: Take 0.2ml iron standard stock solution, add distilled water until volume reaches to 10ml, can be stored at 4℃.

Iron chromogenic agent: Powder A×1 vial, Powder B×1 vial, Liquid C 100ml×1 bottle, can be stored at 4℃ for 6 months. When use, pour Powder A & Powder B in Liquid C, mix sufficiently to dissolve completely, iron chromogenic agent is prepared, it can be stored at 4℃ 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 PRECRDURE

| | Blank tube (O) | Standard tube (S) | Sample tube (U) |
|---|---|---|---|
| Distilled water (ml) | 0.5 | | |
| 2mg/L iron standard working solution (ml) | | 0.5 | |
| Blood serum (ml) | | | 0.5 |
| Iron chromogenic agent (ml) | 1.5 | 1.5 | 1.5 |
| Mix sufficiently, place in boiling water bath for 5 minutes (blank tube and standard tube needn't boiling wate r bath), cool to room temperature, centrifugate at 3500rpm for 10 minutes, take 1.0ml supernatant, transfer i n cuvettes of 0.5cm light path, measure OD values of all tubes at 520nm (adjust zero by double distilled wat | | | |

er).

**5. CALCULATION**

(1) Formula:

$$\text{Blood serum iron content (mg/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (2mg/L)}$$

$$\text{Blood serum iron content (μmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81μmol/L)}$$

* Iron content in standard tube is 2000 g/L, iron's atomic weight is 55.847, so iron content in standard tube is 35.81 mol/L

(2) Example:

Take 0.5ml blood serum to measure iron content, in results, ODBlank is 0.000, ODStandard is 0.038, ODSample is 0.037, calculate as follows:

$$\text{Blood serum iron content (μmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81μmol/L)}$$

$$= \frac{0.037 - 0}{0.038 - 0} \times 35.81 = 34.87(\mu mol/L)$$

6. ANNOUNCEMENTS

Glass wares must be cleansed strictly in order to avoid iron pollution, it is suggested to use disposable plastic test tubes.

If supernatant is turbid, then please transfer it in another test tube and centrifugate again.