

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

# **Ferrous Iron Assay Kit**

Catalog No.: BC00075

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

□ Email (Techsupport) techsupport@enkilife.com

Website: www.enkilife.com

**Shelf life:** Please refer to the label on the outer package.

**Techsupport:** In order to provide you with better service, please inform us the lot number on the label of the outer package.

#### **Basic Information**

Product Name	Ferrous Iron Assay Kit	
<b>Detection Method</b>	Colorimetric	
Sample Type	Serum, plasma, animal tissue, cells	
Assay Type	Quantitative	
<b>Detection Instrument</b>	Microplate reader (593 nm)	

### **Product Introduction**

Iron is one of the essential trace elements for the human body and plays an important role in maintaining normal physiological functions of the body. Ferrous ions are important components of hemoglobin, myoglobin, cytochrome and other enzyme systems, helping oxygen transport and promoting fat oxidation. Iron deficiency can easily cause anemia, metabolic disorders, and affect the body's immune function. Excessive iron is a risk factor for inducing or exacerbating a variety of chronic diseases (such as diabetes, cardiovascular and cerebrovascular diseases, neurodegenerative diseases, etc.).

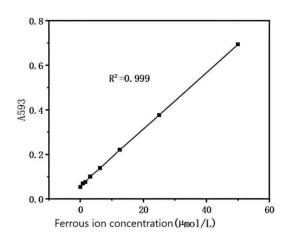
### **Product Features**

• This kit is convenient and quick. The absorbance measurement can be carried out 3-5 minutes after adding the sample to be tested. Usually 10-20 samples can be tested in more than ten minutes.

## **Principle**

Under acidic conditions,  $Fe^{2+}$  forms a blue complex with tripyridyl triazine, which has an absorption peak at 593 nm. The concentration of  $Fe^{2+}$  can be calculated by measuring the absorbance at this wavelength.

The following standard curve is for reference only:



## **Components**

No.	Components	Size (100T)	Storage
Reagent 1	Detection Buffer	20 mL	-20°C, protect from light, store at 2-8°C after opening.
Reagent 2	Color Development Solution	10 mL	-20°C, protect from light, store at 2-8°C after opening.
Reagent 3	Ferrous Ion Standard	1mL/vial, 2 vials	-20°C, protect from light, store at -20°C after opening.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

## **Storage**

The unopened kit can be stored at -20°C for 12 months. After opening, it can be stored at 2-8 °C for 6 months.

## **Preparation**

### Sample handling

- 1. Liquid samples such as serum and plasma: If the serum sample is turbid, it can be diluted with detection buffer before use.
- 2. Tissue sample: Take 0.1g of fresh tissue block, add 1ml of detection buffer to homogenize, centrifuge at 10000×g, 4 °C for 10min, and take the supernatant for later use.

Note: Do not use iron utensils to handle or transfer samples.

### Preparation of the kit

- 1. Equilibrate the reagents in the kit to room temperature.
- 2. Dilution of different concentrations of standards: First, dilute the 10mmol/L ferrous ion standard to 100 μmol/L with the detection buffer, and then dilute it to different concentrations such as 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, and 0 (blank well) μmol/L using the detection buffer according to the half-dilution method.

## **Operation process**

 Standard wells: Take 200 μL of different concentrations of standards and add them to the corresponding standard wells;

Measurement wells: Take 200  $\mu L$  of sample to be tested and add to the corresponding measurement wells.

- 2. Add 100 μL of color development solution to each well in step (1) and mix by gently pipetting.
- 3. Measure the OD value using a microplate reader at 593 nm.

Note: When adding reagents to the ELISA wells, they should be added to touch the bottom of the ELISA plate; add samples slowly to avoid bubbles (bubbles will affect the measurement results).

The operation table is as follows:

	Standard tube (well)	Measurement tube (well)
Different concentrations of ferrous ion standards (µL)	200	
Sample to be tested (µL)		200
Color development solution	100	100

Mix by gently pipetting and oscillating, and measure the OD value at 593 nm using a microplate reader.

### Calculation

Standard fitting curve: y = ax + b

The calculation formula of ferrous ion concentration in the sample is:

Fe<sup>2+</sup>Content ( $\mu$ mol/L) = ( $\Delta$ A<sub>593</sub> - b) ÷ a × f

y: standard OD value - blank OD value

x: concentration of the standard

a: slope of the curve

b: intercept of the curve

ΔA<sub>593</sub>: Sample OD value - Blank OD value

f: dilution factor of the sample before adding it to the detection system

### **Notes**

- 1. Reagents that are blue or nearly blue under acidic conditions will interfere with the detection of this kit and should be avoided as much as possible.
- 2. Substances that affect redox reactions, such as DTT and mercaptoethanol, should not be added to the sample, and detergents such as Tween, Triton and NP-40 should not be added.
- 3. The measurement requires an ELISA reader capable of measuring A593 or a spectrophotometer capable of measuring trace samples.
- 4. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
- 5. The final experimental results are closely related to the effectiveness of the reagents, the relevant operations of the experimenter, the experimental environment and other factors. Our company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible usage of the sample before use and reserve sufficient samples.
- This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.