

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

## Total Iron Assay Kit

Catalog No.: BC00077

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 (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 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

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

## Product Introduction

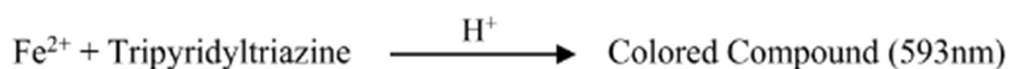
Iron is one of the essential trace elements for the human body and is crucial for physiological processes such as oxygen transport and fat oxidation. The determination of iron ion content is of great significance for evaluating nutritional status, diagnosing iron metabolism-related diseases, and studying the role of iron in biological processes.

## 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 the action of the reducing agent, the iron ions in the sample are reduced to ferrous ions.  $\text{Fe}^{2+}$  forms a blue complex with tripyridyl triazine under acidic conditions, with an absorption peak at 593nm. The content of  $\text{Fe}^{2+}$  can be calculated by measuring the absorbance at this wavelength.



## Components

No.	Components	Size (100T)	Storage
Reagent 1	Detection Buffer	20 mL	-20°C, protected from light, store at 2-8°C after opening.
Reagent 2	Color Development Solution	6 mL	-20°C, protected from light, store at 2-8°C after opening.
Reagent 3	Reducing Agent	6 mL	-20°C, protected from light, store at 2-8°C after opening.
Reagent 4	10mmol/L Iron Ion Standard	1mL/vial, 2 vials	-20°C, protected from light, store at 2-8°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 iron 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, and 0 (blank well) µmol/L using the detection buffer according to the half-dilution method.

## Operation process

1. Standard wells: Take 100µL of different concentrations of standards and add them to the corresponding wells of the microplate;  
Measurement wells: Take 100µL of the sample to be tested and add it to the corresponding wells of the microplate.
2. Add 50 µL of reducing agent to each well in step (1).
3. Add 50 µL of color development solution to each well in step (1) and mix by gently pipetting.
4. Measure the OD value at 593nm using a microplate reader.

**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 iron ion standards (µL)	100	--
Sample to be tested (µL)	--	100
Reducing agent (µL)	50	50
Color development solution (µL)	50	50
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 for total iron ion concentration in the sample is:

$$\text{Fe content } (\mu\text{mol/L}) = (\Delta A_{593} - b) \div a \times f$$

y: standard OD value - blank OD value

x: concentration of the standard

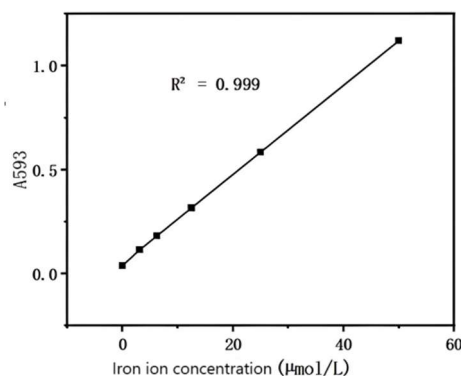
a: slope of the curve

b: intercept of the curve

$\Delta A_{593}$ : Sample OD value - Blank OD value

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

The following standard curve is for reference only:



## 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. Do not use iron utensils to handle or transfer samples.
3. Substances that affect redox reactions, such as DTT and mercaptoethanol, should not be added to the sample, nor should detergents such as Tween, Triton, and NP-40 be added. Metal chelators such as EDTA should not be present in the sample to avoid affecting the color development of the color developer and ferrous ions.
4. The measurement requires an ELISA reader capable of measuring A593 or a spectrophotometer capable of measuring trace samples.
5. 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.
6. 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.
7. 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.