PRODUCT INFORMATION & MANUAL

Ferrous Iron (Fe2+) Assay Kit (Colorimetric)

NBP3-25791

For research use only.

Not for diagnostic or therapeutic procedures.

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Ferrous Iron (Fe2+) Assay Kit (Colorimetric)

Catalog No: NBP3-25791

Method: Colorimetric method

Specification: 100Assays (Can detect 96 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.08 mg/L

Detection range: 0.08-20 mg/L

Average intra-assay CV (%): 3.0

Average inter-assay CV (%): 3.2

Average recovery rate (%): 97

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure the concentration of ferrous iron in serum, plasma and tissue samples.

▲Background

Serum iron is an essential element in human body. Iron with physiological activity mainly exists in the form of ferroheme and transferrin in plasma. 65% of the iron in the body is bound up in hemoglobin molecules in red blood cells. About 4% is bound up in myoglobin molecules. Around 30% of the iron in the body is stored as ferritin or hemosiderin in the spleen, the bone marrow and the liver. Small amounts of iron can be found in other molecules in cells throughout the body. None of this iron is directly accessible by testing the serum. However, some iron is circulating in the serum. Transferrin is a molecule produced by the liver that binds one or two iron (III) ions, i.e. ferric iron, Fe^{3+}. Transferrin is essential if stored iron is to be moved and used.

▲Detection principle

Under the action of acidic solution and reductant, ferric ions can be separated from transferrin in serum, and reduced into ferrous ions (Fe^{2+}). The latter then bind to bipyridine and form pink complexes. The concentration of iron can be calculated by measuring the OD value at 520 nm indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	$FeSO_4 \cdot 7H_2O$ Power	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 2	Chromogenic Agent A	Powder × 4 vials	2-8℃ , 12 months, shading light
Reagent 3	Chromogenic Agent B	50 mL × 4 vials	2-8℃ , 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (520 nm), Test tubes, Vortex Mixer, Centrifuge, Water bath, Test tubes, Vortex Mixer, Centrifuge, Water bath

Reagents:

Deionized water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. Centrifuge after boiling in the water bath, the supernatant must be clarified, otherwise the experimental results will be too biased.
2. Sample tube must boil in the water bath, standard tube and blank tube don't need to.
3. 2 mg/L standard application solution must be prepared as needed. It is recommended that samples be processed before standard preparation.

Pre-assay preparation

▲Reagent preparation

1. Preparation of 100 mg/L standard stock solution:
   Dissolve 1 vial of reagent 1 with deionized water to a final volume of 20 mL. The prepared solution can be stored at 2-8℃ for 1 h.
2. Preparation of 2 mg/L standard working solution:
   Mix the 100 mg/L Standard stock solution and deionized water at a ratio of 1:49. Prepare the fresh needed amount solution before use.
3. Preparation of chromogenic agent:
   Dissolve 1 vial of reagent 2 with 50 mL of reagent 3 fully. The prepared solution can be stored at 2-8℃ for 1 month with shading light.

▲Sample preparation

1. Serum (plasma):

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. The serum must be centrifuged before test if it is turbid. Place the serum on ice for detection.

2. Tissue sample:

Accurately weigh the tissue sample, add 9 times the volume of deionized water according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Sample requirements

Metal chelating agents such as EDTA and citrate should not be added to the samples. The sample should be fresh.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.08-20 mg/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human plasma	1
Human serum	1
10% Rat liver tissue homogenate	1
10% Epipremnum aureum homogenate	1

Note: The diluent is deionized water .

Assay protocol

▲Detailed operating steps

1. Blank tube: Add 0.5 mL of deionized water into a 5 mL centrifuge tube.
   Standard tube: Add 0.5 mL of 2 mg/mL standard working solution into a 5 mL centrifuge tube.
   Sample tube: Add 0.5 mL of sample into a 5 mL centrifuge tube.
2. Add 1.5 mL of chromogenic agent, mix fully with vortex mixer, then incubate in 100℃ water bath for 5 min. (Blank tube and standard tube can be treated without 100℃ water bath.)
3. Cool the tubes with running water, centrifuge the tubes at 2300 g for 10 min. (If the supernatant is still turbid, take the turbid supernatant into another centrifuge tube and centrifuge again.)
4. Take 1.0 mL of supernatant. Set to zero with deionized water , and measure the OD value of each tube with spectrophotometer at 520 nm with 0.5 cm optical path quartz cuvette.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Deionized water (mL)	0.5		
2 mg/L Standard working solution (mL)		0.5	
Sample (mL)			0.5
Chromogenic agent (mL)	1.5	1.5	1.5
Mix fully with vortex mixer, then incubate in 100℃ water bath for 5 min. Cool the tubes with running water, centrifuge the tubes at 2300 g for 10 min. Take 1.0 mL of supernatant. Set to zero with deionized water , and measure the OD value of each tube with spectrophotometer at 520 nm with 0.5 cm optical path quartz cuvette.			

▲Calculation

1.Serum (plasma) sample:

$$\text{Ferrous iron content (mg/L)} = (\Delta A_1) \div (\Delta A_2) \times c_1 \times f$$

or

$$\text{Ferrous iron content (μmol/L)} = (\Delta A_1) \div (\Delta A_2) \times c_2 \times f$$

2. Tissue sample:

$$\text{Ferrous iron content (mg/gprot)} = (\Delta A_1) \div (\Delta A_2) \times c_1 \times f \div C_{pr}$$

or

$$\text{Ferrous iron content (μmol/gprot)} = (\Delta A_1) \div (\Delta A_2) \times c_2 \times f \div C_{pr}$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c_1: The concentration of standard, 2 mg/L

c_2: The concentration of standard, 35.8 μmol/L

2 mg/L standard = 2000 μg/L ÷ Molecular weight of Iron (55.847) = 35.8 μmol/L

f: Dilution factor of sample before test.

C_{pr}: The concentration of protein in sample, gprot/L

Appendix I Data

▲Example analysis

Take 0.5 mL of Human serum, and carry the assay according to the operation table. The results are as follows: the OD value of the standard tube is 0.034, the OD value of the blank tube is 0.004, the OD value of the sample tube is 0.030, and the calculation result is:

$$\text{Ferrous iron content (mg/L)} = \frac{0.030 - 0.004}{0.034 - 0.004} \times 2 = 1.73 \text{ mg/L}$$

Appendix II References

1. Nicolas G, Bennoun M, Porteu A, et al. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proceedings of the National Academy of Sciences of the United States of America, 2002, 99(7): 4596-4601.

2. Conrad M E, Umbreit J N. Iron absorption and transport-An update. American Journal of Hematology, 2000, 64(4): 287-298.

3. Worwood M. Serum ferritin. Clinical Science, 1986, 70(3): 215-220.

4. Emerit J, Beaumont C, Trivin F. Iron metabolism, free radicals, and oxidative injury. Biomedicine & Pharmacotherapy, 2001, 55(6): 333-339.3.