Instructions for Use

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abbexa®

Iron Assay Kit

Catalog No.: abx294097

Size: 100 tests

Detection Range: 0.072 mg/L – 60 mg/L

Sensitivity: 0.072 mg/L

Storage: Store Chromogenic Reagent A and Chromogenic Reagent B in the dark at 2-8°C and the rest of the components at 2-8°C.

Application: For measurement of iron concentration in serum, tissue and other biological fluids.

Introduction

Iron is an essential nutrient essential for the production of hemoglobin, a protein which carries oxygen around in the blood. Iron is also important for the formation of myoglobin which is a protein found in muscle cells, which stores and releases oxygen. Iron deficiency can lead to anemia, cognitive impairments and a weakened immune system. Iron is also involved in metabolism and DNA synthesis. Ferric ions can be separated from transferrin in serum to be reduced into ferrous ions (Fe^{2+}). The ferrous ions can bind to bipyridine and form pink complexes.

Abbexa's Iron Assay Kit is a quick, convenient, and sensitive method for measuring iron concentration. The product has an absorbance maxima at 520 nm. The concentration of iron can be calculated by measuring the OD value indirectly.

Kit components

1. 10 mg/L Iron Standard: 2 ml
2. Chromogenic Reagent A: 4 vials
3. Chromogenic Reagent B: 4 vials
4. Chromogenic Reagent C: 4 x 50 ml

Materials Required But Not Provided

1. Spectrophotometer (520 nm)
2. Deionized water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator
10. Quartz cuvettes

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use.

- **2 mg/L Iron Standard Working Solution**: Mix 10 µl of 10 mg/L Iron Standard and 490 µl of deionized water to create 2 mg/L Iron Standard Working Solution. Prepare 500 µl of 2 mg/L Iron Standard Working Solution for each well. Store at 2-8°C for up to 3 days
- **Iron Chromogenic Reagent**: Dissolve 1 vial of Chromogenic Reagent A and 1 vial of Chromogenic Reagent B with 20 ml of Chromogenic Reagent C and mix well. Store at 2-8°C in the dark for up to 1 month.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 60 mg of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment procedure.

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

Sample Type	Dilution Factor
Human Serum	1
Mouse Serum	1
10% Mouse Liver Tissue Homogenate	1
10% Rat Kidney Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue

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homogenates.

- The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

B. Assay Procedure

1. Set blank, standard and sample tubes. *Pipette samples up and down to mix before adding to tubes. Avoid foaming or bubbles.*
2. Add 0.5 ml of deionized water into the blank tube.
3. Add 0.5 ml of 2 mg/L Iron Standard Working Solution into the standard tube.
4. Add 0.5 ml of sample into the sample tube.
5. Add 1.5 ml of Iron Chromogenic Reagent to all tubes, and mix fully with a vortex mixer.
6. Incubate the sample tubes in a 100°C water bath for 5 minutes. After 5 minutes, cool the sample tubes with running water.
7. Centrifuge all tubes at 2300 x g for 10 minutes. Take 1.0 ml of the supernatant for each tube to measure in the spectrophotometer.
8. Set the spectrophotometer to zero with deionized water.
9. Measure the OD value of each tube with a spectrophotometer at 520 nm with a 0.5 cm optical path.

C. Calculation of Results

1. Serum and plasma samples

$$\text{Iron Content }(mg/L) = \frac{\Delta A_1}{\Delta A_2} \times c_1 \times f \quad \text{or} \quad \text{Iron Content }(\mu\text{mol/L}) = \frac{\Delta A_1}{\Delta A_2} \times c_2 \times f$$

2. Tissues samples:

$$\text{Iron Content }(\text{mg/gprot}) = \frac{\Delta A_1}{\Delta A_2} \times c_1 \times \frac{f}{C_{pr}} \quad \text{or} \quad \text{Iron Content }(\mu\text{mol/gprot}) = \frac{\Delta A_1}{\Delta A_2} \times c_2 \times \frac{f}{C_{pr}}$$

where:

ΔA_1	OD_{Sample} - OD_{Blank}
ΔA_2	$OD_{Standard}$ - OD_{Blank}
c_1	Concentration of the standard (2 mg/L)
c_2	Concentration of the standard (35.8 μmol/L): 2 mg/L Iron Standard is equal to 5.8 μmol/L (2000 μg/L ÷ Molecular weight of Iron (55.847)).
f	The dilution factor of sample
C_{pr}	The concentration of protein in the sample (gprot/L)

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