abbexa®

Iron Assay Kit

Catalog No.: abx298872

Size: 96 tests (80 samples)

Detection Range: 0.29 mg/L - 10 mg/L

Sensitivity: 0.08 mg/L

Storage: Store all components at 2-8°C for up to 6 months.

Application: For detection and quantification of Iron in serum and tissue samples.

For Reference Only

Introduction

Iron is an essential element, forming a major component of hemoglobin, which transports oxygen around the body. Elevated iron concentrations in the blood are associated with various diseases and disorders such as hemochromatosis, hemorrhage, acute hepatitis, defects in iron storage (e.g. pernicious anemia) and certain sideroblastic anemias. Low blood iron concentrations are associated with insufficient iron in diet, chronic blood loss and iron-deficiency anemia.

Abbexa's Iron Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Iron concentrations. Ferric ions upon separation from transferrin in serum, reduced to Ferrous ions which further reacts with bipyridine to produce a pink colored complex. The absorbance should be measured at 520 nm. The intensity of the color is proportional to the concentration of Iron, which can then be calculated.

Kit components

1. 96-well microplate
2. Chromogenic Agent A: 2 vials
3. Chromogenic Agent B: 2 vials
4. Chromogenic Agent C: 20 ml × 2 vials
5. Iron standard (10 mg/L): 2 vial
6. Plate sealer: 2

Materials Required But Not Provided

1. Spectrophotometer or Microplate reader (520 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator
7. Centrifuge
8. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. 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 the tissue homogenate. For each 1 g of homogenate, add 9 ml PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay 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 cell lysates and tissue homogenates.

2. Reagents

- **Chromogenic agent working solution** Dissolve a vial of Chromogenic agent A and a vial of Chromogenic agent B with 20 ml of Chromogenic agent C. Prepare immediately before carrying out the assay. Unused Enzyme working solution can be stored in dark at 2-8°C for up a month.

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B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 1, 2, 4, 5, 6, 8, 10 mg/L. Dilute the 10 mg/L standard stock solution with double distilled water to concentrations of 1, 2, 4, 5, 6, 8, 10 mg/L. The double distilled water itself serves as the 0 mg/L (blank) standard.

2. **Chromogenic Reaction:**

2.1. Set the Standard and Sample tubes.
2.2. Add 75 µl of prepared standard to the Standard tube.
2.3. Add 75 µl of Sample to the Sample tube.
2.4. Add 300 µl of Chromogenic agent working solution to each tube.
2.5. Mix fully with vortex mixer, and then Incubate at 100°C in water bath for 5 minutes.
2.6. After cooling the tubes under running water, centrifuge at 3000 g for 10 minutes.
2.7. Take 200 µl of Standard and Sample supernatants.
2.8. Measure the OD values at 520 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{520} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve (*y* = a*x* + b). The Iron concentration of the samples can be interpolated from the standard curve.

1. Iron concentration in Serum samples:

$$\text{Iron (mg/L)} = \frac{\Delta A_{520} - b}{a} \times f$$

2. Iron concentration in Tissues samples:

$$\text{Iron (mg/g protein)} = \frac{\Delta A_{520} - b}{a} \times \frac{f}{C_P}$$

where:

ΔA_{520}	OD value of the standard ($OD_{Sample} - OD_{Blank}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
C_P	concentration of protein in sample (g protein/L)

For Reference Only