Total Iron Binding Capacity (TIBC) Assay Kit

Catalog No.: abx298948

Size: 96 tests

Sensitivity: 0.14 mg/l
Detection Range: 0.31-50 mg/l

Storage: Store the Iron Standard stock solution, Reagent A and Reagent B in the dark at 4°C and the rest of the components in the dark at 4°C for up to 6 months.
Application: For quantification of Total Iron Binding Capacity (TIBC) in serum.

Introduction

Iron is added to the serum sample, which binds to bind all the ferritin present in the serum, and excess iron is adsorbed by adding the iron adsorbent. Iron bound to ferritin is separated from other proteins in the sample by the addition of acid solution and reducing agent. Fe3+ is reduced to Fe2+, which binds with bipyridine to form a pink complex. The intensity of the color is proportional to the TIBC. By colorimetric analysis of the bipyridine-Fe2+ complex, the units of Total Iron Binding Capacity can be tested.

Abbexa's Total Iron Binding Capacity (TIBC) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating TIBC. The bipyridine-Fe2+ complex has an absorbance maxima at 520 nm. The intensity of the color is proportional to the TIBC, which can then be calculated.

Kit components

1. 96-well microplate
2. Reagent A: 2 vials
3. Reagent B: 2 vials
4. Reagent C: 2 x 15ml
5. 100mg/L Iron Standard Stock Solution: 1 x 2ml
6. Iron Absorbent: 79 vials
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (510-530 nm)
2. Double-distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Water bath
6. Centrifuge
7. Vortex mixer
8. Incubator

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.

1. **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. Shortly before assay, pretreat the sample: Add 50 µl of serum, and 50 µl of 10 mg/L Iron Standard solution to an EP tube. Vortex and stand at room temperature for 5 minutes. Add one vial of Iron Absorbent, vortex for 3 seconds, then stand at room temperature for 5 minutes. Centrifuge at 3000g for 10 minutes and take the supernatant (pretreated sample) for detection. If precipitation occurs, centrifuge again.

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

Sample Type	Dilution Factor
Human Serum	1
Rat Serum	1
Porcupine Serum	1
Rabbit Serum	1
Chicken Serum	1
Machin Serum	1

Note:

- The dilutant is double distilled water
- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

2. Reagents

- **All reagents:** Bring to room temperature before use
- **Reagents A, B and C:** Dissolve **Reagent A** and **Reagent B** together with 15mL of **Reagent C** fully to produce one vial of **Reagent Working Solution**. Can be stored at 4°C for a month in the dark.

B. Assay Procedure

2. Set control, control blank, standard, standard blank, sample and sample blank wells on the microplate and record their positions. It is recommended to use 2 control, 2 control blank, 2 standard and 2 standard blank wells for each assay run. Each sample requires a sample blank well. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
3. Add 100mg/L Iron Standard Stock Solution serial dilutions with double distilled water. Recommended dilutions (0, 5, 10, 20, 25, 30, 40, 50 mg/L)
4. Add 30 µl of each dilution of the Iron Standard Stock Solution to the standard and standard blank wells.
5. Add 150 µl of Reagent Working Solution (see A2. Reagents) to each standard well
6. Sample tube: Add 250 µl of Reagent Working Solution and 50 µl of pretreated sample to an EP tube
   Control tube: Add 250 µl of Reagent Working Solution and 50 µl of double distilled water to an EP tube
7. Vortex the Sample tube and Control tube for 3 seconds, and heat at 100°C in a water bath for 5 minutes
8. Cool each tube with cold running water, then centrifuge at 10000g for 10 minutes. If precipitation occurs, centrifuge again.
9. Take 180 µl of the supernatant for each tube, and add to the corresponding microplate wells.
10. Measure the OD of each well with a microplate reader at 520 nm.

C. Calculation of Results

1. Serum

- The standard curve can be plotted as the relative OD of each reference standard solution (X), against the respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor, to obtain the concentration before dilution.

$$\text{Total Iron Binding Capacity (TIBC, mg/L)} = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{ControlBlank}}) - b}{a \times f}$$

$$\text{Total Iron Binding Capacity (TIBC, µmol/L)} = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{ControlBlank}}) - b}{a \times f \times \text{C}_{\text{Standard}}}$$

$$\text{Unsaturated Iron Binding Capacity (UIBC, µmol/L)} = \text{TIBC (µmol/L)} - \text{C}_{\text{Serum}}\text{(µmol/L)}$$

$$\text{Iron Saturation (i, \%)} = \frac{\text{C}_{\text{Serum}}\text{(µmol/L)}}{\text{TIBC (µmol/L)}} \times 100$$

Where:

Y= aX + b is the equation of the standard curve

a = The slope of the standard curve

b = The intercept of the standard curve

f = Dilution factor of the sample before test

$C_{Standard}$ = Standard concentration = 1 mg/L = 17.91µmol/L

C_{Serum} = Serum Iron concentration