Total Iron Binding Assay Kit

Catalog No.: abx294056

Size: 50 tests

Detection Range: 0.03 mg/L – 50 mg/L

Sensitivity: 0.03 mg/L

Storage: Store all components at 4°C. Store Chromogenic Reagent A and B in the dark.

Application: For detection and quantification of Total Iron Binding Capacity concentrations in serum samples.

Introduction

Abbexa's Total Iron Binding Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Iron Binding Capacity (TIBC) concentration. Iron is added to the serum in excess to bind to all the ferritin in the serum. The unbound iron is then adsorbed by adding an iron adsorbent. The iron that is bound with the ferritin protein is separated from the ferritin using an acidic solution and reductant. The Fe3+ that is released in the serum is reduced to Fe2+. Fe2+ can then bind with bipyridine to form a pink-colored complex which has an aborbance at 520 nm. The amount of TIBC is positively correlated with the measured absorbance at 520 nm. The iron content measured can also be used to calculate the unsaturated iron binding capacity (UIBC) by adjusting for the sample iron concentration.

Kit components

1. Chromogenic Reagent A: 2 vials
2. Chromogenic Reagent B: 2 vials
3. Chromogenic Reagent C: 2 × 60 ml
4. Standard (100 mg/L): 7 ml
5. Iron Adsorbant: 50 vials

Materials required but not provided

1. Spectrophotomer (520 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Water bath
8. Vortex mixer

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Samples

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

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

- **Serum:** Add 1 ml of sample into 1ml of prepared Standard (**10 mg/L**) Solution and mix thoroughly. Leave to stand at room temperature for 10 minutes. Add one vial of Iron Adsorbant and mix thoroughly. Leave to stand at room temperature for 5 minutes. Continue to mix and leave to stand 4 times. Centrifuge at 2300 × g for 10 minutes. Carefully take the supernatant and assay immediately.

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 double-distilled water or normal saline (0.9 % NaCl), 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
Rat serum	1
Porcine serum	1
Rabbit serum	1
Chicken serum	1
Monkey (Maqaque) serum	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **Chromogenic Reagent Working Solution:** Add 1 vial of Chromogenic reagent A and one vial of Chromogenic Reagemnt B into 60 ml of Chromogenic Reagent C and mix thoroughly to dissolve. Unused Chromogenic Reagent Working Solution can be aliquot and stored for up to 1 month at 4°C in the dark.
- **Standard (10 mg/L) Solution:** Prepare enough Standard (10 mg/L) Solution for each sample used in the assay. Per sample, add 100 µl of Standard (100 mg/L) and 900 µl of double-distilled water and mix thoroughly to prepare 1000 µl of Standard (10 mg/L) Solution. Unused Standard (10 mg/L) Solution can be stored for up to 3 days at 4°C.

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- **Standard (1 mg/L) Solution:** Prepare enough Standard (1 mg/L) Solution for each standard tube used in the assay. Per tube, add 100 µl of Standard (10 mg/L) Solution and 900 µl of double-distilled water and mix thoroughly to prepare 1000 µl of Standard (1 mg/L) Solution. Unused Standard (1 mg/L) Solution can be stored for up to 3 days at 4°C.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- After incubating the tubes in the water bath at 100°C, the supernatant collected after centrifugation must be clear to avoid affecting the experimental results.
- Ensure all experimental containers are clean before use to avoid the contamination of iron.

B. Assay Procedure

1. Mark microcentrifuge tubes for each standard, sample, and blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 1 ml of sample to each sample tube.
3. Add 1 ml of Standard (1 mg/L) Solution to the standard tube.
4. Add 1 ml of double-distilled water to the blank tube.
5. Add 2 ml of Chromogenic Reagent Working Solution to each tube. Mix thoroughly using a vortex mixer then incubate in a water bath at 100°C for 5 minutes
6. Cool the tubes with running water then centrifuge at 2300 × g for 10 minutes. If the supernatant is not clear, carefully take the supernatant to a new tube and centrifuge further. Carefully take 1 ml of supernatant.
7. Set the spectrophotomometer to zero using double-distilled water.
8. Measure the OD of each tube at 520 nm with a 0.5 cm optical path cuvette.

C. Calculation of Results

1. Serum samples:

$$\text{Total Iron Binding Capacity (mg/L)} = \mathbf{F} \times \mathbf{C_1} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}})}{(\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}})}$$

Or

$$\text{Total Iron Binding Capacity (µmol/L)} = \mathbf{F} \times \mathbf{C_2} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}})}{(\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}})}$$

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$$\text{Unsaturated Iron Binding Capacity (µmol/L)} = \text{TIBC - } C_3$$

$$\text{Iron Saturation (\%)} = \frac{C_3}{\text{TIBC}} \times 100$$

where:

OD_{Sample}	OD value of sample
$OD_{Standard}$	OD value of standard
OD_{Blank}	OD value of blank
C_1	Concentration fo standard (1 mg/L)
C_2	Concentration of standard (17.91 µmol/L)
C_3	Sample iron concentration (µmol/L)
TIBC	Total Iron Binding Capacity (µmol/L measurement)
F	The dilution factor of sample
Molar Concentration of Iron	1mg/L Iron = 1000 µg/L ÷ Mr of Iron (55.847) = 17.91 µmol/L

For Reference Only

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.