Total Iron-binding Capacity Microplate Assay Kit User Manual

Catalog # FTA0219

(Version 1.2A)

Detection and Quantification of Total Iron-binding Capacity (TIBC) in Serum, Plasma Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Total iron-binding capacity (TIBC) or sometimes transferrin iron-binding capacity is a medical laboratory test that measures the blood's capacity to bind iron with transferrin. It is performed by drawing blood and measuring the maximum amount of iron that it can carry, which indirectly measures transferrin since transferrin is the most dynamic carrier.

Total Iron-binding Capacity Microplate Assay Kit is a sensitive assay for determining total Iron-binding capacity in various samples. The intensity of the product color, measured at 562 nm, is proportional to the total Iron-binding capacity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	16 ml x 1	4 °C
Assay Buffer II	6 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 4 ml distilled water to dissolve before use.

Dye Reagent A: add 6 ml distilled water to dissolve before use.

Dye Reagent B: add 6 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 10 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 562 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. SAMPLE PREPARATION

1. For liquid samples

Detect directly, or dilute with distilled water.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Control	Standard	Blank
Reaction Buffer I	80 µl	80 µl	80 µl	80 µl
Sample	20 µl	20 µl	--	--
Standard	--	--	20 µl	--
Distilled water	--	--	40 µl	60 µl
Substrate	20 µl	20 µl	--	--
Mix, incubate at 37 °C for 10 minutes.				
Dye Reagent A	30 µl	30 µl	30 µl	30 µl
Dye Reagent B	30 µl	30 µl	30 µl	30 µl
Distilled water	20 µl	--	--	--
Reaction Buffer II	--	20 µl	--	--
Mix, incubate at 37 °C for 5 minutes, record absorbance measured at 562 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The total iron-binding capacity can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

1. According to the protein concentration of sample

$$TIBC\ (\mu mol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 10 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the volume of sample

$$TIBC\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 10 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

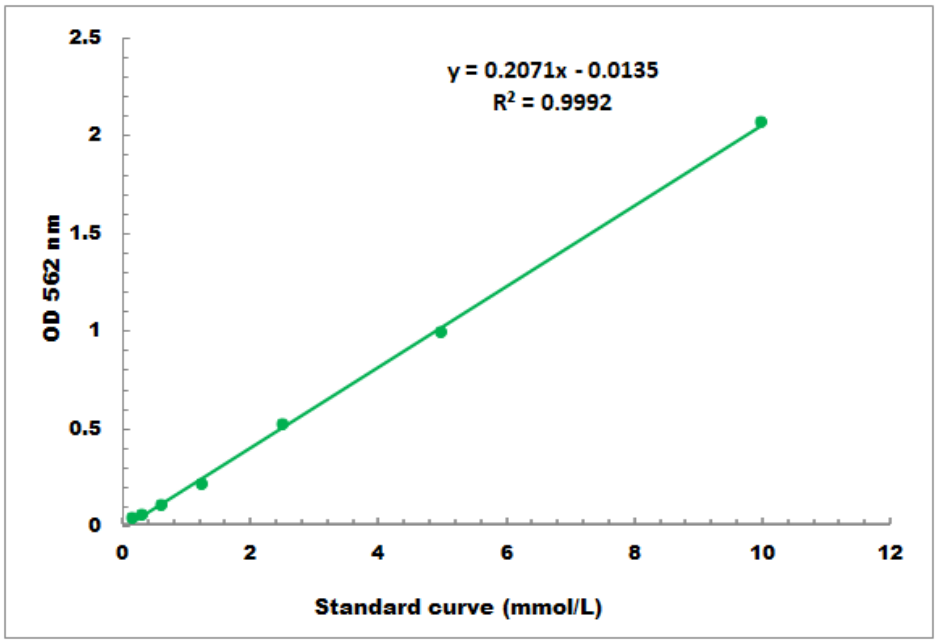
$C_{Standard}$: the standard concentration, 10 mmol/L = 10 μmol/ml;

V_{Sample}: the volume of sample, 0.02 ml;

$V_{Standard}$: the volume of standard, 0.02 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES