



# Total Iron-binding Capacity Microplate Assay Kit

**Catalog # AS0191**

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
VI. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

## 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 mlx 1	4 °C
Assay Buffer II	6 mlx 1	4 °C
Substrate	Powderx 1	4 °C
Dye Reagent A	Powderx 1	4 °C
Dye Reagent B	Powderx 1	4 °C
Standard	Powderx 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 10mmol/L.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 562 nm
2. Distilled water
3. 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 $\mu$ l	80 $\mu$ l	80 $\mu$ l	80 $\mu$ l
Sample	20 $\mu$ l	20 $\mu$ l	--	--
Standard	--	--	20 $\mu$ l	--
Distilled water	--	--	40 $\mu$ l	60 $\mu$ l
Substrate	20 $\mu$ l	20 $\mu$ l	--	--
Mix, incubate at 37°C for 10 minutes.				
Dye Reagent A	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l
Dye Reagent B	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l
Distilled water	20 $\mu$ l	--	--	--
Reaction Buffer II	--	20 $\mu$ l	--	--
Mix, incubate at 37°C for 5 minutes, record absorbance measured at 562 nm.				

## VI. CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned} \text{TIBC } (\mu\text{mol/mg}) &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ & \quad (\text{V}_{\text{Sample}} \times \text{C}_{\text{Protein}}) \\ &= 10 \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{C}_{\text{Protein}} \end{aligned}$$

2. According to the volume of sample

$$\begin{aligned} \text{TIBC } (\mu\text{mol/ml}) &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{V}_{\text{Sample}} \\ &= 10 \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$\text{C}_{\text{Protein}}$ : the protein concentration, mg/ml;

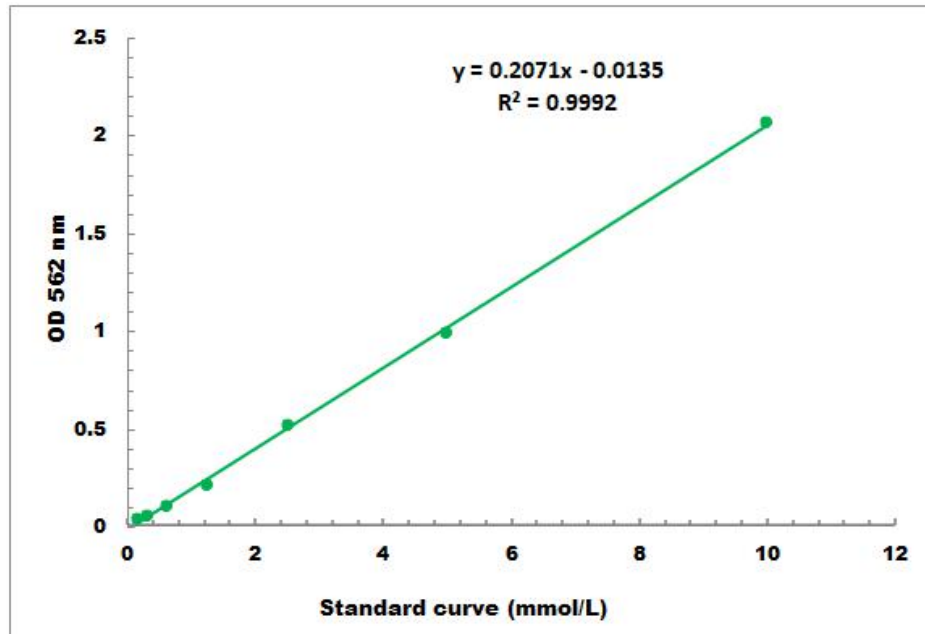
$\text{C}_{\text{Standard}}$ : the standard concentration, 10 mmol/L = 10 $\mu$ mol/ml;

$\text{V}_{\text{Sample}}$ : the volume of sample, 0.02 ml;

$\text{V}_{\text{Standard}}$ : 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: 0.1mmol/L -10mmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.sabbiotech.cn](http://www.sabbiotech.cn) or contact us at [techcn@signalwayantibody.com](mailto:techcn@signalwayantibody.com)

## IX. NOTES