Total Iron-binding Capacity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1242

(Version 1.4A)

Detection and Quantification of Total Iron-binding Capacity(TIBC) inSerum, 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 Colorimetric Microplate Assay Kitis 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	
Reaction Buffer I	16 mlx 1	4 °C
Reaction Buffer II	6 mlx 1	4 °C
Substrate	Powderx 1	4 °C, keep in dark
Dye Reagent A	Powderx 1	4 °C, keep in dark
Dye ReagentB	Powderx 1	4 °C, keep in dark
Standard	Powderx 1	4 °C, keep in dark
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. Centrifuge
6. Timer
7. Hot air circulation oven

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 mldistilled waterto generate 10 mmol/L of standardtop solution,keep in dark and store at 4 °C for 1 day or -20°C for 2-3 monthsafter reconstitution.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Substrate: Add 4 ml distilled water to dissolve before use.Keep in dark and store at 4 °C for 1 day or -20°C for 2-3 monthsafter reconstitution.

Dye Reagent A: Add 6 ml distilled water to dissolve before use.Keep in dark and store at -20°C for 2-3 weeksafter reconstitution.

Dye Reagent B: Add 6 ml distilled water to dissolve before use.Keep in dark and store at 4 °C for 3-4days or -20°C for 1 monthafter reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For liquid samples

Detect directly, or dilute with distilled water.

VI. 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 at37°C for 10minutes.				
Dye Reagent A	30µl	30µl	30µl	30µl
Dye Reagent B	30µl	30µl	30µl	30µl
Distilled water	20 µl	--	--	--
Reaction Buffer II	--	20 µl	--	--
Mix, incubate at37°C for 5minutes, recordabsorbance measured at 562 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations 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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Control}-OD_{Sample})-\text{Intercept}}{\text{Slope}}\times n(\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

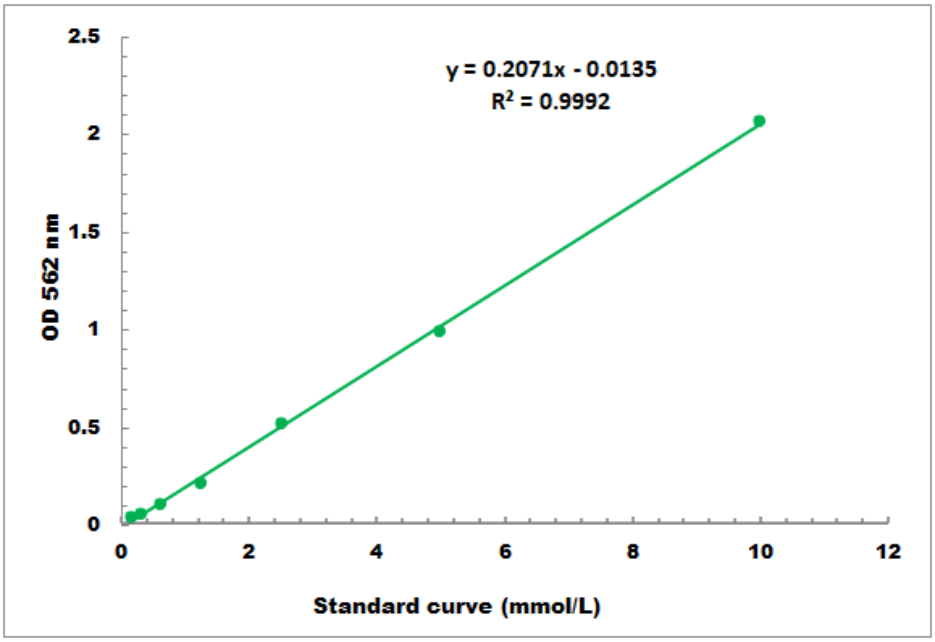
$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

VIII. 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