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# CheKine™ Micro Total Iron-Binding Capacity (TIBC) Assay Kit

Cat #: KTB2150

Size: 96 T/96 S

[ <u>;</u> ]	Micro Total Iron-Binding Capacity (TIBC) Assay Kit		
REF	<b>Cat #</b> : KTB2150	LOT	Lot #: Refer to product label
	Detection range: 15-2000 µmol/L		Sensitivity: 15 µM
	Applicable samples: Serum, Plasma		
X	Storage: Stored at 4°C for 6 months, protected from light		

# **Assay Principle**

Total Iron-Binding Capacity (TIBC) refers to the ability of serum transferrin to combine iron, and its content is closely related to the occurrence of iron deficiency anemia, acute hepatitis and other diseases. CheKine<sup>TM</sup> Micro Total Iron-Binding Capacity (TIBC) Assay Kit provides a simple method for detecting TIBC concentration in serum and plasma sample. The principle is that  $Fe^{2+}$  reacts with ferrozine to form a purply-red compound with characteristic absorption peak at 562nm. Serum transferrin can be bonded to  $Fe^{3+}$  under alkaline conditions, and the remaining unbound  $Fe^{3+}$  can be reduced to  $Fe^{2+}$ , at this point, the absorbance A<sub>1</sub> is positively correlated with the amount of unbound  $Fe^{3+}$ . The bonded  $Fe^{3+}$  could be released after acidification, and then be reduced into  $Fe^{2+}$ , at this point, absorbance A<sub>2</sub> is positively correlated with the total amount of  $Fe^{3+}$ . And A<sub>2</sub> minus A<sub>1</sub> is proportionate to the concentration of TIBC.

# **Materials Supplied and Storage Conditions**

	Size	- Storage conditions
Kit components	96 T	
Reagent I	34 mL	4°C
Reagent II	5 mL	4°C, protected from light
Reagent III -A	2.5 mL	4°C, protected from light
Reagent III -B	2.5 mL	4°C, protected from light
Reagent∣∨	7.5 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

# Materials Required but Not Supplied

• Microplate reader or visible spectrophotometer capable of measuring absorbance at 562 nm

· 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips



- Water bath
- · Deionized water

#### **Reagent Preparation**

**Reagent I**: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent II : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Reagent III: Before use, mix Reagent III-A and Reagent III-B according to the ratio of 1:1. Prepare and use immediately.

ReagentIV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Note: Reagent II is corrosive, please take protective measures when operating.

### **Sample Preparation**

Serum: Tested directly.

#### **Assay Procedure**

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 562 nm, visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement (the following operations are operated in the EP tube).

Reagent	Blank Tube (µL)	Test Tube (μL)			
Serum	0	40			
Deionized Water	80	0			
Reagent I	280	280			
Reagent II	0	40			
Mix well, incubate in 37°C for 10 min.					
ReagentIII	40	40			
Mix well, incubate in 37°C for 5 min, and take 200 µL to the 96-well plate or microglass cuvette. The absorbance value is					

measured at 562 nm. The test well is marked as  $A_1$ , and the blank well is marked as  $A_3$ . Then add Reagent  $|| \vee ||$  immediately after the measurement.

Mix well, incubate in 37°C for 5 min. The absorbance value is measured at 562 nm with a microplate reader. The test well is marked as  $A_2$ , and the blank well is marked as  $A_4$ , Finally calculate  $\Delta A_{\text{Test}} = (A_2 - A_1) - (A_4 - A_3)$ .

Note: Blank well only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If  $\Delta A_{Test}$  is greater than 1.5, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor.

### **Data Analysis**

TIBC definition: At 37°C, the number of µmol of Fe<sup>3+</sup> bound to each L of serum.

TIBC (μmol/L)=(697×ΔA<sub>Test</sub>+35.1)×n

n: Sample dilution factor.

# **Typical Data**

Take 40 µL rabbit serum, follow the determination steps, and measure with 96-well plate:



$$\begin{split} A1=&0.68, A2=&0.848, A3=&0.779, A4=&0.799, \Delta A_{test}=&(A_2-A_1)-(A_4-A_3)=&(0.848-0.68)-(0.799-0.779)=&0.148;\\ Calculate\ TIBC:\ TIBC\ (\mu mol/L)=&(696.6\times\Delta A_{Test}+35.1)\times1=&(696.6\times0.148+35.1)\times1=&138.2\ \mu mol/L. \end{split}$$

### **Recommended Products**

Catalog No.	Product Name
KTB2100	CheKine™ Micro Serum Potassium (K⁺) Assay Kit
KTB2130	CheKine™ Micro Serum Sodium Assay Kit
KTB2140	CheKine™ Micro Serum Zinc Assay Kit
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit

### **Disclaimer**

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

