



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

Cat #: KTB2150

Size: 96 T

	Micro Total Iron-Binding Capacity (TIBC) Assay Kit		
REF	Cat #: KTB2150	LOT	Lot #: Refer to product label
	Detection range: 15-2000 µmol/L		Sensitivity: 15 µmol/L
	Applicable samples: Serum		
	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™ Micro Total Iron-Binding Capacity (TIBC) Assay Kit provides a simple method for detecting TIBC concentration in serum sample. The principle is that Fe²⁺ reacts with ferrozine to form a purply-red compound with characteristic absorption peak at 562nm. Serum transferrin can be bonded to Fe³⁺ under alkaline conditions, and the remaining unbound Fe³⁺ can be reduced to Fe²⁺, at this point, the absorbance A₁ is positively correlated with the amount of unbound Fe³⁺, The bonded Fe³⁺ could be released after acidification, and then be reduced into Fe²⁺, at this point, absorbance A₂ is positively correlated with the total amount of Fe³⁺. And A₂ minus A₁ is proportionate to the concentration of TIBC.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Reagent I	30 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 IV	7 mL	4°C

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. Store at 4°C, protected from light.

Reagent IV: 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.

Reagent III	40	40
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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 A₁, and the blank well is marked as A₃. Then add Reagent IV immediately after the measurement.

Reagent IV	60	60
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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 A₂, and the blank well is marked as A₄. 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 ΔA_{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 Fe³⁺ bound to each L of serum.

$$\text{TIBC } (\mu\text{mol/L}) = (697 \times \Delta A_{\text{Test}} + 35.1) \times n$$

n: Sample dilution factor.

Typical Data

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

$$A_1 = 0.68, A_2 = 0.848, A_3 = 0.779, A_4 = 0.799, \Delta A_{\text{test}} = (A_2 - A_1) - (A_4 - A_3) = (0.848 - 0.68) - (0.799 - 0.779) = 0.148;$$

Calculate TIBC: $TIBC (\mu\text{mol/L}) = (696.6 \times \Delta A_{\text{Test}} + 35.1) \times 1 = (696.6 \times 0.148 + 35.1) \times 1 = 138.2 \mu\text{mol/L}$.

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.