EZMeta™ Total Iron-Binding Capacity (TIBC) Colorimetric Assay Kit

Cat #: D-AKC2150

Size: 96T

Storage: Stored at 4°C for 6 months, protected from light

Product Information

Detection range: 15-2000 μmol/L

Sensitivity: 15 μmol/L

Applicable samples: Serum

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. EZMeta™ Total Iron-Binding Capacity (TIBC) Colorimetric Assay Kit provides a simple method for detecting TIBC concentration in serum 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 Fe2+, at this point, the absorbance A_1 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_2 is positively correlated with the total amount of Fe^{3+}. And A_2 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.

ReagentⅢ: Before use, mix ReagentⅢ-A and ReagentⅢ-B according to the ratio of 1:1. Store at 4°C, protected from light. **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 ReagentⅣ immediately after the measurement.		
ReagentⅣ	60	60
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 $\Delta A_{Test}=(A_2-A_1)-(A_4-A_3)$.		

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 Fe^{3+} bound to each L of serum.

TIBC (μmol/L)**=(697×ΔA_{Test}+35.1)×n**

n: Sample dilution factor.

Typical Data

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

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 (μmol/L)=(696.6×ΔA_{Test}+35.1)×1=(696.6×0.148+35.1)×1=138.2 μmol/L.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.