GENLISA® Total Iron-Binding Capacity (TIBC) Assay

Cat No: KBCA2261

Pack Size: 96T (79 samples)

Measuring instrument：Microplate reader (510-530 nm)
Detection range：0.31-50 mg/l

Intended use

This kit can be used to measure the total iron binding capacity (TIBC) content in serum samples.

Detection principle

The excess iron is added to the serum to bind all the ferritin in the serum, and the excess iron is adsorbed by adding the iron adsorbent. The iron bind with the ferritin is separated from the protein by the action of acid solution and reductant. Fe3+ in serum is reduced to Fe2+, Fe2+ binds with bipyridine to form pink complex. In a certain range, the amount of TIBC is positively correlated with the depth of color. The iron content measured is, minus serum iron value, which is called unsaturated iron binding force. Total iron binding capacity minus serum iron value is unsaturated iron binding capacity (UIBC).

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	100 mg/l Iron Standard Stock Solution	2 ml × 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent A	Powder × 2 vials	2-8°C, 12 months shading light
Reagent 3	Chromogenic Agent B	Powder × 2 vials	2-8°C, 12 months shading light
Reagent 4	Chromogenic Agent C	15 ml x 2 vials	2-8°C, 12 months
Reagent 5	Iron Absorbent	Powder x 79 vials	2-8°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (510-530 nm, optimum wavelength: 520 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge.

Reagents:

Double distilled water.

Reagent preparation

1. Equilibrate all the reagents to room temperature before use.
2. The preparation of chromogenic agent:
   Dissolve 1 vial of chromogenic agent A and 1 vial of chromogenic agent B with 15 ml of chromogenic agent C, mix well to dissolve. Store at 2-8°C for 1 month protected from light.
3. The preparation of standard curve :
   Always prepare a fresh set of standards. Discard working standard dilutions after use.
   Dilute 100 mg/l iron standard stock solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 40, 50 mg/l. Reference is as follows:

Item	1	2	3	4	5	6	7	8
Concentration (mg/l)	**0**	**5**	**10**	**20**	**25**	**30**	**40**	**50**
100 mg/l standard (ul)	0	10	20	40	50	60	80	100
Double Distilled Water (ul)	200	190	180	160	150	140	120	100

Sample preparation

1. Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80^{0}C for a month.

2. Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Rat plasma	1
Porcine serum	1
Rabbit serum	1
Chicken serum	1
Machin Serum	1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

(1) After 100ºC water bath, the supernatant obtained by centrifugation must be clarified, otherwise the experimental results will be affected.
(2) The experimental container must be clean to avoid the contamination of iron.

Operating steps

The measurement of standard curve:

1. Dilute 100 mg/l iron standard stock solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 40, 50 mg/l

2. Take 30 ul of standard solution with different concentration to the wells.

3. Add 150 ul of chromogenic agent to the wells.

4. Mix fully for 5s with microplate reader, stand at room temperature for 5 min and measure the OD value at 520 nm.

The measurement of sample:

1. The pretreatment of sample:

   Take 50 ul of serum, add 50 ul of 10 mg/l iron standard application solution, mix fully with a vortex mixer and stand at room temperature for 5 min. Then add a vial of iron absorbent, mix fully with a vortex mixer for 3 s and stand at room temperature for 5 min. Centrifuge at 3000×g for 10 min and take the supernatant for detection.

2. Sample tube: Add 50 ul of pretreated sample into the 1.5 ml EP tube.
   Control tube: Add 50 ul of double distilled water into the 1.5 ml EP tube.

3. Add 250 ul of chromogenic agent into each tube. Oscillate fully with a vortex mixer for 3 s and incubate in 100ºC water bath for 5 min.

4. Cool the tubes with running water, then centrifuge at 10000×g for 10 min (If the supernatant is turbid, collect the turbid supernatant into another new EP tube and centrifuge again).

5. Take 180 ul of the supernatant to the corresponding wells of microplate and measure the OD value at 520 nm of each well.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard # 1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (Plasma) Sample:

$$\text{TIBC (mg/l)} = (\Delta A_{520} - b) \div a \times f$$

OR

$$\text{TIBC (umol/L)} = (\Delta A_{520} - b) \div a \times f \times c_1$$

$$\text{UIBC (umol/L)} = c_3 - c_2$$

$$i = c_2 \div c_3 \times 100\%$$

[Note]

f: Dilution factor of sample before test.
ΔA_{520}: $OD_{Sample} - OD_{Blank}$.
c_1: 17.91 umol/L (1 mg/l Iron = 17.91 umol/L).
c_2: The concentration of serum iron.
c_3: Total iron binding capacity (TIBC) (umol/L).
i: Iron saturation (%).

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/l)	3.80	24.60	43.80
%CV	1.7	1.6	1.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/l)	3.80	24.60	43.80
%CV	2.5	2.1	2.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/l)	8.5	22.6	32
Observed Conc. (mg/l)	8.6	22.4	32.0
Recovery rate (%)	101	99	100

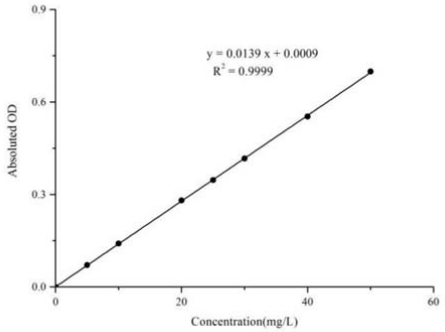
Sensitivity

The analytical sensitivity of the assay is 0.14 mg/l. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (mg/l)	0	5	10	20	25	30	40	50
Average OD	0.037	0.108	0.178	0.318	0.384	0.454	0.590	0.736
Absoluted OD	0	0.071	0.141	0.281	0.347	0.417	0.553	0.699



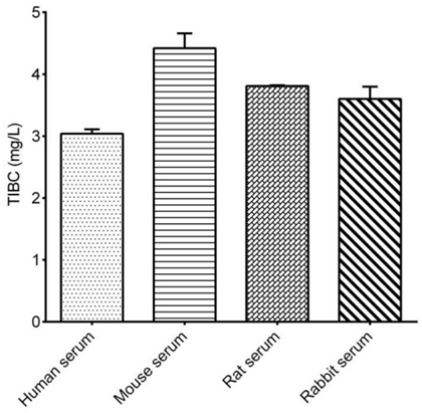
Appendix II Example Analysis

Example analysis :

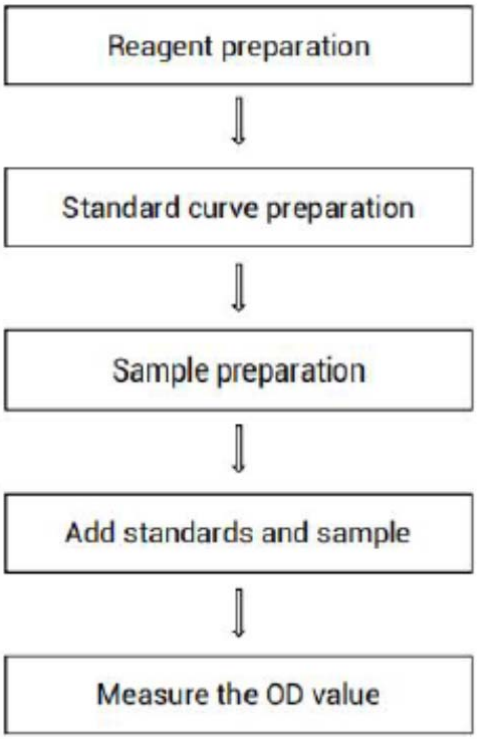
Take 50 ul of human serum and carry the assay according to the operation steps. The results are as follows: Standard curve: y = 0.014 x – 0.0009, the average OD value of the sample is 0.080, the average OD value of the control is 0.038, and the calculation result is:

TIBC = (0.080 - 0.038 + 0.0009) ÷ 0.014 = 3.06 mg/l (mg/l)

Detect human serum, mouse serum, rat serum and rabbit serum according to the protocol, the result is as follows :



Assay Summary



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