Total Bilirubin (TBIL) Colorimetric Assay Kit

Catalog No: E-BC-K760-M

Method: Colorimetric method

Specification: 96T (Can detect 46 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.7 μmol/L

Detection range: 0.7-50 μmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used for detection of total bilirubin (TBIL) content in serum sample.

▲Background

Bilirubin is one of the important components of bile. It is the degradation product of hemoglobin in various heme proteins under the action of a series of enzymes. It is important to the digestion and absorption of lipids and the formation of yellow distemper. Bilirubin comes in two forms: water-soluble and water-insoluble. Bilirubin has powerful antioxidant, anti-inflammatory and autoimmune properties. The concentration of bilirubin in human body is related to sex, drug intake, age and so on. Low serum bilirubin is directly related to diabetes, metabolic syndrome, cardiovascular disease and other pathological states. However, high bilirubin is indicative of hemolysis, jaundice, Gilbert syndrome, hepatitis, drug toxicity, and possible bile duct obstruction.

▲Detection principle

Under the action of accelerant, the hydrogen bond in indirect bilirubin is broken, which makes the insoluble indirect bilirubin and direct bilirubin react with azo reagent to form azo bilirubin under acidic conditions. The azo bilirubin generated has the maximum absorption at 565 nm. The content of total bilirubin in serum can be obtained by measuring the change of absorbance.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Acid Agent	30 mL × 1 vial	2-8℃，6 months, shading light
Reagent 2	Diazonium Salt	10 mL × 1 vial	2-8℃，6 months
Reagent 3	Stop Solution	5 mL × 1 vial	2-8℃，6 months, shading light
Reagent 4	Standard	Powder × 2 vials	2-8℃，6 months, shading light
Microplate		96 wells	No requirement
Plate Sealer		2 pieces	
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.			

▲Materials prepared by users

Instruments

Microplate reader (565nm) , Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. When adding samples, add them quickly or use multiple-channel pipettes.
2. There should be no bubbles in the wells of the microplate when measuring the OD value.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of working solution:
   Mix reagent 1 and reagent 2 at a ratio of 1.2:1 fully. Prepare the fresh needed amount solution before use.
3. Preparation of 25 μmol/L standard solution:
   Dissolve reagent 4 with 2 mL of double distilled water, mix fully and store it with shading light. Prepare the needed amount before use and preserve it on ice for detection.

▲Sample preparation

1. Serum sample: Detect directly
2. Sample requirements: There is no hemolysis in the serum sample

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.7-50 μmol/LL).

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

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Rat serum	1
Rabbit serum	1
Chicken serum	1
Porcine serum	1

Note: The diluent is normal saline (0.9% NaCl) .

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	565 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A'	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
B	A	A'	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'
C	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
D	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
E	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
F	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
G	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
H	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'

Note: A, standard wells; A', standard $_{control}$ wells; S1-S46, sample wells; S1'-S46', sample $_{control}$ wells.

▲Operation table

1. Standard tube: Take 80 μL of reagent 1 into 0.5 mL EP tube.
   Standard $_{control}$ tube: Take 80 μL of reagent 1 into 0.5 mL EP tube.
   Sample tube: Take 80 μL of reagent 1 into 0.5 mL EP tube.
   Sample $_{control}$ tube: Take 80 μL of reagent 1 into 0.5 mL EP tube.
2. Add 160 μL of working solution into standard tubes and sample tubes.
   Add 160 μL of double distilled water into standard $_{control}$ tubes and sample $_{control}$ tubes.
3. Add 30 μL of 25 μmol/L standard into standard tubes and standard $_{control}$ tubes.
   Add 30 μL of sample into sample tubes and sample $_{control}$ tubes.
4. Mix fully, incubate at 37°C for 5 min.
5. Add 20 μL of reagent 3 into each tube.
6. Mix fully, incubate at 37°C for 5 min. Take 250 μL of reaction solution into the corresponding wells and measure the OD values of each well at 565 nm with microplate reader.

▲Operation table

	Standard tube	Standard$_{control}$ tube	Sample tube	Sample$_{control}$ tube
Reagent 1 (μL)	80	80	80	80
Working solution (μL)	160		160	
Double distilled water (μL)		160		160
Sample (μL)			30	30
25 μmol/L Standard (μL)	30	30		
Mix fully and incubate at 37°C for 5 min.				
Reagent 3 (μL)	20	20	20	20
Mix fully, incubate at 37°C for 5 min. Take 250 μL of reaction solution into the corresponding wells and measure the OD values of each well at 565 nm with microplate reader.				

▲Calculation

$$TBIL\ (\mu mol/L) = A_2 \div A_1 \times C \times f$$

Note:

A_2: the OD value of sample - the OD value of $sample_{control}$

A_1: the OD value of standard- the OD value of $standard_{control}$

C: Concentration of standard (25 μmol/L)

f: Dilution factor of sample before tested

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.7-50 μmol/L	Average intra-assay CV (%)	2.8
Sensitivity	0.7 μmol/L	Average inter-assay CV (%)	4.0
Average recovery rate (%)	96		

▲Example analysis

Take 30 μL of serum and carry the assay according to the operation table.The results are as follows:

The OD value of the sample is 0.094, the OD value of the sample$_{control}$ is 0.063, the OD value of the standard is 0.150, the OD value of the standard $_{control}$ is 0.041, and the calculation result is:

TBIL content (μmol/L)=(0.094-0.063)÷(0.150-0.041)×25=7.11 μmol/L