（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K760-M

Specification：48T(22 samples)/96T(46 samples)

Measuring instrument：Microplate reader（565 nm）

Detection range：0.7-50 μmol/L

Elabscience® Total Bilirubin (TBIL) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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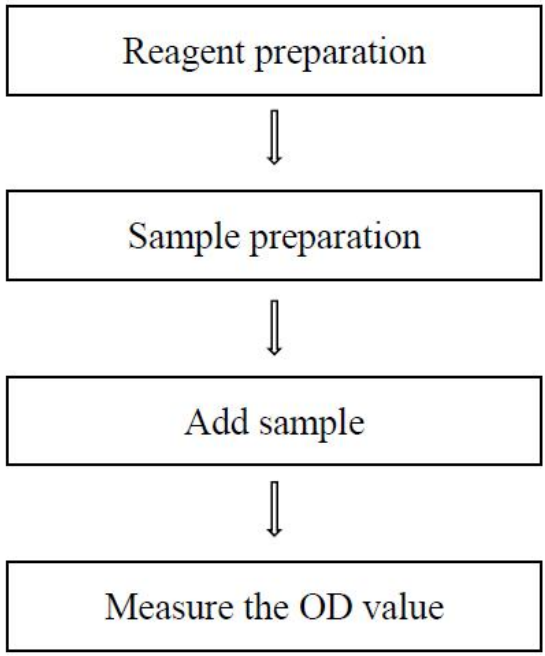
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Acid Agent	15 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Diazonium Salt	5 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 months
Reagent 3	Stop Solution	5 mL × 1 vial	5 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 4	Standard	Powder × 2 vials	Powder × 2 vials	2-8℃, 12 months, shading light
	Microplate	48 wells	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Bring all reagents to room temperature before use.

② Preparation of working solution：

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 165 μL of working solution (mix well 90 μL of acid agent and 75 μL of diazonium salt, mix well. The working solution should be prepared on spot.

③ Preparation of 25 μmol/L standard solution：

Dissolve one vial of standard with 2 mL of double distilled water, mix well to dissolve. The working solution should be prepared on spot and stored protected from light.

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℃ 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 serum	1
Mouse serum	1
Rabbit serum	1
Chicken serum	1
Porcine serum	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

① Standard tube: Take 80 μL of acid agent into 0.5 mL EP tube.
Standard control tube: Take 80 μL of acid agent into 0.5 mL EP tube.
Sample tube: Take 80 μL of acid agent into 0.5 mL EP tube.
Sample control tube: Take 80 μL of acid agent 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℃ for 5 min.

⑤ Add 20 μL of stop solution into each tube.

⑥ Mix fully, incubate at 37℃ 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

The sample:

$$\underset{\mu mol/L}{TBIL} = \frac{A_2}{A_1} \times C \times f$$

[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.

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 (μmol/L)	3.50	22.70	42.00
%CV	3.1	2.8	2.5

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 (μmol/L)	3.50	22.70	42.00
%CV	3.7	4.2	4.1

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 96%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	8.5	26	38.5
Observed Conc. (μmol/L)	8.4	24.4	36.6
Recovery rate (%)	99	94	95

Sensitivity

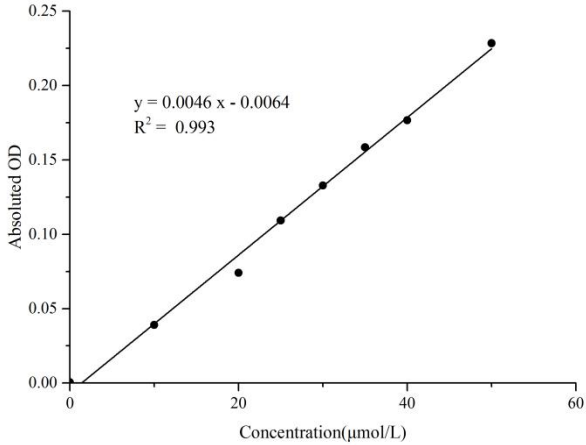
The analytical sensitivity of the assay is 0.7 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 (μmol/L)	**0**	**10**	**20**	**25**	**30**	**35**	**40**	**50**
OD value of standard	0.039	0.079	0.125	0.152	0.176	0.197	0.216	0.274
	0.037	0.079	0.125	0.148	0.173	0.202	0.219	0.271
Average OD	0.037	0.079	0.115	0.150	0.174	0.200	0.217	0.273
OD value of standard$_{control}$	0.037	0.041	0.041	0.041	0.041	0.041	0.041	0.044
	0.038	0.039	0.042	0.040	0.042	0.041	0.041	0.044
Average OD	0.038	0.040	0.041	0.041	0.041	0.041	0.041	0.044
Absoluted OD	0.000	0.039	0.074	0.109	0.133	0.159	0.177	0.229



Appendix II Example Analysis

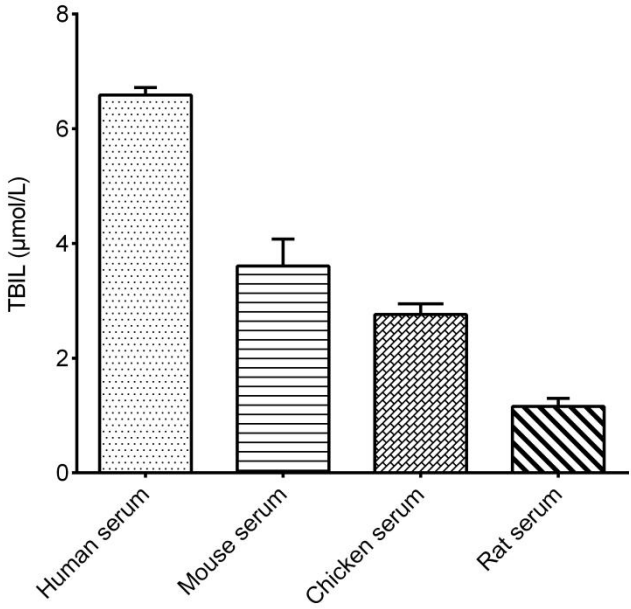
Example analysis：

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

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



Appendix III Publications

1. Yuan J , Ding L , Han L ,et al.Thermal/ultrasound-triggered release of liposomes loaded with Ganoderma applanatum polysaccharide from microbubbles for enhanced tumour ablation[J].Journal of Controlled Release, 2023, 363(000):17.DOI:10.1016/j.jconrel.2023.09.030.
2. Li Y, Li T Y, Qiao Q, et al. Polymeric immunoglobulin receptor promotes Th2 immune response in the liver by increasing cholangiocytes derived IL-33: a diagnostic and therapeutic biomarker of biliary atresia[J]. EBioMedicine, 2024, 108.
3. Zeng Z, Quan C, Zhou S, et al. Gut microbiota and metabolic modulation by supplementation of polysaccharide-producing Bacillus licheniformis from Tibetan Yaks: A comprehensive multi-omics analysis[J]. International Journal of Biological Macromolecules, 2024, 254: 127808.
4. Li Q, Du Y, **ang P, et al. Re-visiting antioxidant therapy in murine advanced atherosclerosis with brussels chicory, a typical vegetable in mediterranean diets[J]. Nutrients, 2023, 15(4): 832.
5. Ma X, Zhang W, Chen Y, et al. Paeoniflorin inhibited GSDMD to alleviate ANIT-induced cholestasis via pyroptosis signaling pathway[J]. Phytomedicine, 2024, 134: 156021.
6. Zhao J Q , Sun Y , Yang L L ,et al.New finding based on Comparative Toxicogenomics Database: Hepatic YY1 mediates drug-induced liver injury[J].Phytomedicine, 2024, 135(000):17.DOI:10.1016/j.phymed.2024.156102.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.