Total Bilirubin (TBIL) Assay Kit (Diazo Method)

Detection instrument: Spectrophotometer/ Microplate reader

Catalog Number: JYM009CHS

Size: 50T/24S、100T/48S

1. **Product Composition**: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact our staff in time.

Reagent name	50T	100T	Preservation Condition
Reagent I	Liquid 10 mL×1	Liquid 20 mL×1	2-8℃
Reagent II	Liquid 4 mL×1	Liquid 6 mL×1	2-8℃
Reagent III	Liquid 1.6 mL×1	Liquid 3 mL×1	2-8℃
Standard	Powder×1	Powder×1	-20℃
96-well plate (Cat#YA0501)	96-well plate × 1	96-well plate×1	-
Sealing Membrane (Cat#YA0249)	2 pieces	2 pieces	-

Solution preparation:

1. **Reference standard:** Bilirubin reference standard.
2. **10 mmol/L bilirubin standard:** Prior to use, add 593μL of distilled water and mix thoroughly to prepare a 10mmol/L bilirubin standard solution for plotting the calibration curve.
3. **Working solution preparation:** Immediately prior to use, prepare the working solution according to the sample volume using a ratio of Reagent I: Reagent II = 1050μL:875μL (total 1925μL, approximately 12 seconds). Prepare and use immediately.

Product Description:

Total Bilirubin (TBIL) is the sum of direct bilirubin and indirect bilirubin Indirect bilirubin is poorly soluble in water and cannot be excreted via the kidneys. Within hepatocytes, it combines with glucuronic acid to form direct bilirubin; direct bilirubin is water-soluble and can be excreted from the body via the kidneys in urine.The liver is responsible for the uptake, conjugation and excretion of bilirubin. Total bilirubin serves as a crucial indicator of liver function and is the primary basis for diagnosing jaundice, aiding in the assessment of hepatic and biliary disorders as well as haematological conditions.

Under acidic conditions, indirect bilirubin is converted to direct bilirubin with the aid of the accelerator CTAB. Direct bilirubin reacts directly with the diazonium reagent (chlorinated diazobenzene sulfonic acid formed from p-aminobenzenesulfonic acid and sodium nitrite) to produce a purple-red azo compound exhibiting a characteristic absorption peak at 530 nm. enabling the calculation of total bilirubin concentration.

Reagents and Equipment Required but Not Provided:

spectrophotometer/microplate reader, Water bath/Metal bath/Constant-temperature incubator, centrifuge, adjustable pipette, Microvolume Glass Cylinder/96-well Plate, ice and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Serum/plasma and other liquids: Direct measurement (if turbid, centrifuge and measure the supernatant).

II. Determination operation

1. Preheat the visible spectrophotometer/microplate reader for at least 30min. Adjust the wavelength to 530nm. Zero the visible spectrophotometer using distilled water.
2. Prior to the experiment, equilibrate Reagent I, Reagent II, and Reagent III to room temperature.
3. Dilution of standard solutions: Dilute the 10mmol/L (equivalent to 10,000μmol/L) bilirubin standard solution with distilled water to prepare stock solutions at concentrations of 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625μmol/L for subsequent use.
4. Standard Sample Dilution Table

Serial number	Concentration prior to dilution (μmol/mL)	Volume of standard sample (μL)	Distilled water volume (μL)	Diluted concentration (μmol/mL)
1	10000	10	900	100
2	100	500	500	50
3	50	500	500	25
4	25	500	500	12.5
5	12.5	500	500	6.25
6	6.25	500	500	3.125
7	3.125	500	500	1.5625

Note: Each standard tube requires 30μL of standard solution(take care not to measure the absorbance value at this stage).

5. Operation table(Add reagents into 1.5 mL centrifuge tube with the following list):

Reagent name(μL)	Test tube (T)	Control tube(C)	Standard tube(S)	Blank control (B)
Reagent I	80	80	80	80
working fluid	160	-	160	160
distilled water	-	160	-	30
Sample	30	30	-	-
Standard	-	-	30	-
Thoroughly mix, react at 37°C **in the dark** for 5min, then immediately add the following reagents:				
Reagent III	20	20	20	20

Thoroughly mix, incubate at 37°C in the dark for 5 min, centrifuge at 12,000rpm at room temperature for 5min. Pipette 200μL of supernatant and measure absorbance A at 530nm. Record as A_T, A_C, A_S, and A_B respectively. Calculate $\Delta A_T = A_T - A_C$ and $\Delta A_S = A_S - A_B$. (The standard curve and blank tubes require measurement only once or twice.)

Note: 1. The experimental procedure must be conducted strictly in the absence of light, and the order of sample addition must not be altered.

2. Should the sample volume be excessive, it is advisable to conduct the analysis in batches.

III. Calculation:

1. Create standard curve

Establish a standard curve based on the concentration (x, μmol/L) and absorbance ΔA standard (y, ΔA standard) of the standard tubes. Using the standard curve, substitute the measured ΔA (y, ΔA measured) into the formula to calculate the sample concentration (x, μmol/L).

2. Calculation of total bilirubin (TBIL) content:

Total bilirubin concentration (μmol/L) = x×F

F: Dilution ratio.

Note

1. If the ΔA measurement exceeds 0.4, dilute the sample with distilled water before analysis. If the ΔA measurement falls below 0.005, increase the sample volume appropriately while reducing the reagent volume by one unit before analysis. Ensure the calibration curve is adjusted accordingly.

Experimental example:

1. Take 10mmol/L bilirubin standard solution and dilute to 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 μmol/L. Measure the absorbance of each standard tube at 30 μL. Plot the standard curve: y =0.0031x- 0.0072, R^2 = 0.9992.

2. The test results for samples of different species are as follows:

Sample	A_T	A_C	ΔA_T	TBIL content	Unit
Bovine serum	0.082	0.058	0.024	10.065	μmol/L
Rabbit serum	0.068	0.059	0.009	5.258	μmol/L
Horse serum	0.087	0.059	0.028	11.258	μmol/L
Rat plasma	0.064	0.060	0.004	3.710	μmol/L
Human serum	0.075	0.059	0.016	7.419	μmol/L

Note: 1. The aforementioned experiment was conducted using a 96-well plate.