Technical Manual

Direct Bilirubin (DBIL) Colorimetric Assay Kit

- Catalogue Code: MAES0194
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.6-50 µmol/L

Sensitivity:

0.6 µmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

This kit can be used for detection of direct bilirubin (DBIL) content in animal serum sample.

4. Detection Principle

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 direct bilirubin in serum can be obtained by measuring the change of absorbance.

5. Kit Components & Storage

Item	Specification	Storage
Acid Agent	30 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Diazonium Salt	10 mL × 1 vial	2-8°C, 6 months
Stop Solution	5 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Centrifuge
- Microplate Reader (565 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal saline (0.9% NaCl)

6. Assay Notes:

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.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **working solution:** Mix acid agent and diazonium salt at a ratio of 1.2：1 fully. Prepare the fresh needed amount solution before use.

3. Preparation of **standard solution (25 μmol/L):** Dissolve standard with 2 mL of double distilled water, mix fully and store it avoiding direct sunlight. Prepare the needed amount before use and preserve it on ice for detection.

8. Sample Preparation

Sample requirements: There is no hemolysis in the serum sample.

Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.6-50 μmol/L).

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).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 565 nm

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.

10. Operation Steps

The measurement of samples

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.

2. **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.

3. Add 160 μL of working solution into standard tubes and sample tubes.

4. Add 160 μL of double distilled water into standard control tubes and sample control tubes.

5. Add 30 μL of 25 μmol/L standard into standard tubes and standard control tubes.

6. Add 30 μL of sample into sample tubes and sample control tubes.

7. Mix fully, incubate at 37°C for 5 min.

8. Add 20 μL of stop solution into each tube.

9. Mix fully, incubate at 37°C for 5 min. Take 250 mL 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
Acid agent (μL)	80	80	80	80
Working solution (μL)	160		160	
Double distilled water (μL)		160		160
Sample (μL)			30	30
Standard (25 μmol/L) (μL)	30	30		
Mix fully and incubate at 37°C for 5 min.				
Stop solution (μL)	20	20	20	20
Mix fully, incubate at 37°C for 5 min. Take 250 mL of reaction solution into the corresponding wells and measure the OD values of each well at 565 nm with microplate reader.				

11. Calculations

$$\text{DBIL content } (\mu\text{mol/L}) = \frac{A_2}{A_1} \times C \times f$$

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

12. Performance Characteristics

Detection Range	0.6-50 μmol/L
Sensitivity	0.6 μmol/L
Average recovery rate (%)	100
Average inter-assay CV (%)	8.6
Average intra-assay CV (%)	4.0

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 0.083, the OD value of the sample control is 0.064, the OD value of the standard is 0.105, the OD value of the standard control is 0.041, and the calculation result is:

$$\textbf{DBIL content } (\mu\text{mol/L}) = (0.083 - 0.064) \div (0.150 - 0.041) \times 25$$

$$= 4.35\ \mu\text{mol/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assaygenie.com