TECHNICAL MANUAL

Direct Bilirubin (DBIL) Colorimetric Assay Kit

- **SKU CODE:** MAES0194
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add acid agent and working solution/water
- Add standard and sample
- Incubate at 37°C for 5 min
- Add stop solution
- Incubate at 37°C for 5 min
- Measure absorbance at 565 nm

2. Intended use

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

3. Detection principle

Direct bilirubin reacts with azo reagent to form azo bilirubin under acidic conditions. The azo bilirubin generated has maximum absorption at 565 nm. The content of direct bilirubin in serum can be obtained by measuring the change in absorbance.

4. 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°C, 12 months, shading light
Reagent 2	Diazonium Salt	5 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months
Reagent 3	Stop Solution	5 mL × 1 vial	5 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Standard	Powder × 2 vials	Powder × 2 vials	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the number of test wells. For example, prepare 165 µL of working solution by mixing 90 µL of acid agent and 75 µL of diazonium salt, mix well. The working solution should be prepared fresh.
3. **Preparation of 25 µmol/L standard solution:** Dissolve one vial of standard with 4 mL of double distilled water, mix well to dissolve completely. The 25 µmol/L standard solution should be prepared fresh and stored protected from light.

7. Sample preparation

1. **Sample preparation:** Serum and plasma: detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one month.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

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

9. 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°C for 5 min.
5. Add 20 µL of stop solution 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.

10. Calculation

The sample:

DBIL (µmol/L) = $(A_2/A_1) \times C \times f$

[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.

11. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	2.50	23.40	45.50
%CV	4.5	4.1	3.4

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	2.50	23.40	45.50
%CV	8.2	8.8	8.8

Recovery

Three samples of high, middle, and low concentration were tested with each concentration assayed 6 times in parallel to get the average recovery rate of 100%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (µmol/L)	12.5	26.5	42
Observed Conc. (µmol/L)	12.4	26.8	42.0
Recovery rate (%)	99	101	100

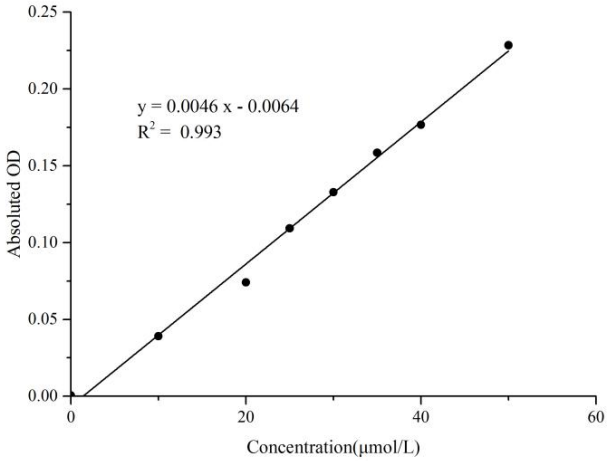
Sensitivity

The analytical sensitivity of the assay is 0.6 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 is not necessary to prepare the standard curve for this kit; 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), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μmol/L)	OD value of standard		Average OD	OD value of standard control		Average OD	Absolute OD
0	0.039	0.038	0.038	0.037	0.038	0.038	0.000
10	0.079	0.039	0.040	0.041	0.039	0.040	0.039
20	0.125	0.042	0.041	0.041	0.042	0.041	0.074
25	0.152	0.040	0.041	0.041	0.040	0.041	0.109
30	0.176	0.042	0.041	0.041	0.042	0.041	0.133
35	0.197	0.041	0.041	0.041	0.041	0.041	0.159
40	0.216	0.041	0.041	0.041	0.041	0.041	0.177
50	0.274	0.044	0.044	0.044	0.044	0.044	0.229

12. Appendix II Example Analysis

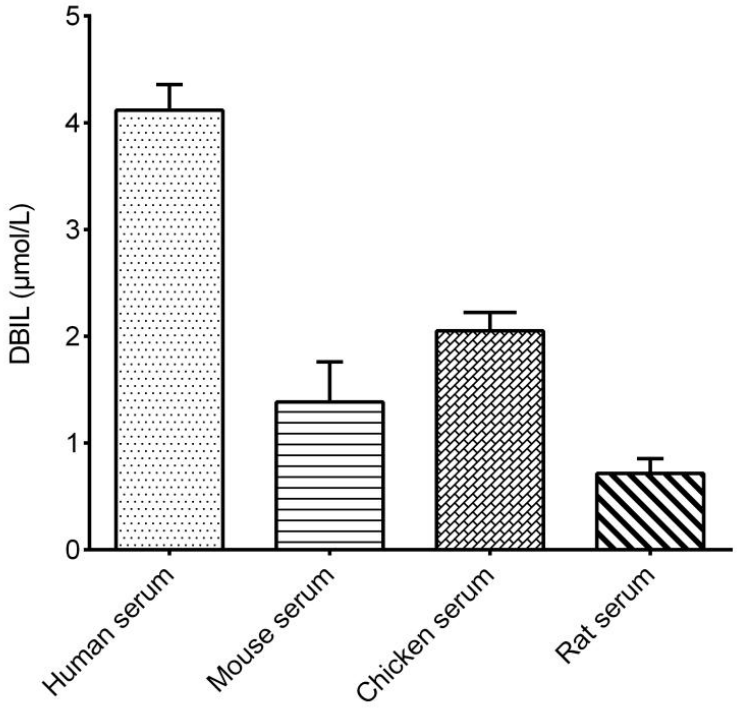
Example analysis:

Take 30 μmol/L of serum and carry out 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:

DBIL content (μmol/L) = (0.083-0.064) ÷ (0.150-0.041) × 25 = 4.35 μmol/L

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



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.