Total Bilirubin (TBIL) Colorimetric Assay Kit

Catalogue No.: K095

Size: 96T(46 samples)

Kit component:

Item	Specifications
Acid Agent	30 mL
Diazonium Salt	10 ml
Stop Solution	5 ml
Standard	Powder × 2 vials
Product Description	1 copy

Storage:

The kit should be stored at 2-8℃ shading light for 6 months.

Principle of the Assay:

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.

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

Assay Procedure (For reference):

1. Preparation of reagents

a. Bring all reagents to room temperature before use.

b. Preparation of working solution: Mix Acid Agent and Diazonium Salt at the volume ratio of 1:2 before use.

c. 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.

2. Preparation of samples

Serum sample: detect directly. No hemolysis is required.

Before the formal test, 2-3 samples with significant differences should be selected and diluted into different concentrations for pre-test. Determine whether dilution is required according to the pre-test results and the kit detection range of 0.7-50 μmol/L. If dilution is required, use normal saline (0.9% NaCl) as diluent.

3. Add samples and test

①Standard 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.

②Add 160 μL of working solution into standard tube and sample tube in step 1.

Add 160 μL of double distilled water into standard control tube and sample control tube in step 1.

③Add 30 μL of 25 μmol/L standard into standard tube and standard control tube in step 2.

Add 30 μL of sample into sample tube and sample control tube in step 2.

④Mix fully, incubate at 37℃ for 5 min.

⑤Add 20 μL of stop solution into each tube.

⑥Mix 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.

4. Calculation

$$\text{Total Bilirubin (TBIL)（μmol/L）} = \frac{A2}{A1} \times C \times f$$

Note:

A2: the OD value of sample - the OD value of sample control.

A1: 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 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 a lab coat and latex gloves during the experiment.

4. The detection range of the kit is not equivalent to the concentration range of the substance to be measured in the sample. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.

5. If the samples to be tested are not among the types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.

6. The experimental results are closely related to the situation of reagents, operations, environment and so on. We 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.