



SUNLONG

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Total bilirubin (TBIL) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: AK0660-100T-96S

Size: 100T/96S

Components:

Reagent I: 30 mL×1. Store at 2-8°C.

Reagent II: 10 mL×1. Store at 2-8°C.

Product Description :

Total bilirubin (TBIL) is the sum of direct bilirubin and indirect bilirubin. Determination of serum total bilirubin is an important item in liver and gallbladder function tests. The content of direct bilirubin is of great significance for clinical diagnosis of latent jaundice. Total bilirubin could be oxidized by sodium nitrite to form biliverdin, which has absorbance in 450 nm. The content of total bilirubin can be calculated by detecting the wavelength change at 450 nm.

Reagents and Equipment Required but Not Provided :

Spectrophotometer/Microplate reader, desk centrifuge, constant temperature foster box/water-bath, pipette, micro glass cuvette/96 well flat-bottom plate, ice and distilled water.

Procedure

I. Sample preparation:

Serum, plasma or other liquid samples: Detect sample directly. If the solution is turbid, perform the measurement after centrifuging.

II. Determination procedure :

- Preheat spectrophotometer/microplate reader for 30min, adjust wavelength to 450 nm, set spectrophotometer counter to zero with distilled water.
- Determination:

Reagent (μL)	Test tube	Blank tube
Sample	20	-
Distilled water	-	20
Reagent I	240	240
Mix well. Avoid light and react at 37°C for 5 minutes. Measure the absorbance at 450 nm, record as A _{1T} , A _{1B} ;		
Reagent II	60	60

Mix well. Avoid light and react at 37°C for 5 minutes. Measure the absorbance at 450 nm, record as A_{2T}, A_{2B}. Calculate the $\Delta A_T = A_{1T} - A_{2T}$, and the $\Delta A_B = A_{1B} - A_{2B}$. Blank tube only need to be measured once or twice.

(when first step of 5min reaction completed and colorimetric in cuvette completed, the reagent II can be directly added to the cuvette for uniform reaction for 5min)

III. Calculations :

A. 96 well flat-bottom plate

$$\text{TBIL content } (\mu\text{mol/L}) = 874.67 \times (\Delta A_T - \Delta A_B) + 10.699$$

B. Micro glass cuvette

$$\text{TBIL content } (\mu\text{mol/L}) = 491.98 \times (\Delta A_T - \Delta A_B) + 18.478$$

Note:

1. Bilirubin decomposes easily in light. Avoid light during measurement.
2. If the $\Delta A < 0.01$, it is recommended to increase the sample size before determination; If $\Delta A > 1.5$, it is recommended to dilute the sample before determination.

Examples:

1. Take mouse serum to follow the determination procedure to operate. Determination with 96 well flat-bottom plate, and calculate $\Delta A_T = A_{1T} - A_{2T} = 0.278 - 0.148 = 0.13$, $\Delta A_B = A_{1B} - A_{2B} = 0.051 - 0.048 = 0.003$.

The calculated content is as follows:

$$\text{TBIL content } (\mu\text{mol/L}) = 874.67 \times (\Delta A_T - \Delta A_B) + 10.699 = 121.78 \mu\text{mol/L}.$$