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CheKine™ Micro Total Bilirubin (TBIL) Content Assay Kit

Cat #: KTB1412 Size: 48 T/48 S 96 T/96 S

	Micro Total Bilirubin (TBIL) Content Assay Kit		
REF	**Cat #:** KTB1412	LOT	**Lot #:** Refer to product label
	Detection range: 3.4-476 μmol/L		**Sensitivity:** 3.4 μmol/L
	Applicable samples: Serum, Plasma		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Total bilirubin is the sum of direct bilirubin and indirect bilirubin. Elevated total bilirubin in serum (plasma) is commonly found in hepatitis, extrahepatic biliary obstruction, hemolytic diseases, etc. It is an important biochemical item for clinical liver and gallbladder function testing. The CheKine™ Micro Total Bilirubin (TBIL) Content Assay Kit measures the total bilirubin content in serum or plasma. Its principle is that total bilirubin is oxidized in the presence of surfactants and oxidants to produce biliverdin. The decrease in absorbance at 450 nm is directly proportional to the concentration of total bilirubin, and the content of total bilirubin in serum (plasma) can be calculated through changes in absorbance.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	12 mL	24 mL	4℃
Reagent Ⅱ	3 mL	6 mL	4℃
Standard	Powder×2 vial	Powder×2 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- water bath
- Deionized water

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard: Before use, a standard was dissolved in 0.2 mL of deionized water at a concentration of 36.0 μmol/L. The dissolved standard is unstable and can only be stored at -20℃ and use on the same day.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

Serum (plasma) sample: Test directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Deionized water	20	0	0
Standard	0	20	0
Sample	0	0	20
Reagent I	200	200	200
Mix well and incubate at 37℃ for 5 min. Measure the absorbance A_1 at 450 nm, recording the values as $A_{1\ Blank}$, $A_{1\ Standard}$, and $A_{1\ Test}$, respectively.			
Reagent II	50	50	50

Mix well and incubate at 37℃ for 5 min. Measure the absorbance A_2 at 450 nm, recording the values as $A_{2\ Blank}$, $A_{2\ Standard}$, and $A_{2\ Test}$, respectively. Calculate $\Delta A_{Blank}=A_{1\ Blank}-A_{2\ Blank}$, $\Delta A_{Standard}=A_{1\ Standard}-A_{2\ Standard}$, $\Delta A_{Test}=A_{1\ Test}-A_{2\ Test}$, $\Delta\Delta A_{Standard}=\Delta A_{Standard}-\Delta A_{Blank}$, $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$.

Note: The Blank Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, it is recommended to do a pre-experiment with 2-3 samples. If the ΔA_{Blank} is greater than 0.05, it cannot be used and should be discarded. If $\Delta\Delta A_{Test}$ is less than 0.006, increase the sample quantity appropriately. If the concentration of TBIL in the sample is greater than 476 μmol/L, the sample can be further diluted with deionized water, and the calculated result can be multiplied by the dilution factor. Bilirubin is easily decomposed when exposed to light, so it should be measured in the dark as much as possible. During sample testing, $A_{1\ Test}$ and $A_{2\ Test}$ are commonly found to be similar, indicating an extremely low bilirubin content in the sample. If the $\Delta\Delta A_{Test}$ is negative, it indicates that bilirubin has been degraded and the sample cannot be used, it is recommended to resample for testing.

Data Analysis

TBIL(μmol/L)=$\Delta\Delta A_{Test}\div\Delta\Delta A_{Standard}\times C_{Standard}\times n$

$C_{Standard}$: Concentration of Standard, 36.0 μmol/L; n: Sample dilution factor.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1690	CheKine™ Micro Gamma-Glutamyl Transpeptidase (γ-GT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.