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Direct bilirubin (DIBL) Content Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: AK0622-100T-96S

Size: 100T/96S

Components:

Reagent I: 25 mL×1. Store at 2-8℃.

Reagent II: 6 mL×1. Store at 2-8℃.

Product Description：

Direct bilirubin (DBIL) is also called conjugated bilirubin. After indirect bilirubin enters the liver, it is combined with glucuronic acid by the action of glucuronosyltransferase in the liver. The increase of direct bilirubin is of great significance for clinical diagnosis of obstructive jaundice, hepatocellular jaundice, liver cancer, pancreatic head cancer, cholelithiasis and cholangiocarcinoma. Direct bilirubin could be oxidized by sodium nitrite to form biliverdin, which has absorbance in 450 nm. The content of direct 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：

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

Reagent (μL)	Test tube	Blank tube
Sample	8	-
Distilled water	-	8
Reagent I	192	192
Mix well. React at 37℃ for 5 minutes. Measure the absorbance at 450 nm, record as $A1_T$, $A1_B$;		
Reagent II	48	48

Mix well. React at 37℃ for 5 minutes. Measure the absorbance at 450 nm, record as $A2_T$, $A2_B$. Calculate the $\Delta A_T = A1_T - A2_T$, and the $\Delta A_B = A1_B - A2_B$. 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

DBIL content (μmol/L) $=1495.7\times(\Delta A_T-\Delta A_B)-15.343$

B. Micro glass cuvette

DBIL content (μmol/L) $=1099.7\times(\Delta A_T-\Delta A_B)-10.73$

Note:

1. Bilirubin decomposes easily in light. Avoid light during measurement.
2. If the ΔA is lower, it is recommended to increase the sample size before determination; If $\Delta A > 0.5$, 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 $\Delta A_T = A1_T - A2_T = 0.217 - 0.170 = 0.047$, $\Delta A_B = A1_B - A2_B = 0.044 - 0.044 = 0$. The calculated content is as follows:

   DBIL content (μmol/L) $=1495.7\times(\Delta A_T-\Delta A_B)-15.343=54.95$ μmol/L.