

# Direct bilirubin (DIBL) Content Assay Kit

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

**Operation Equipment:** Spectrophotometer/Microplate reader

**Catalog Number:** BC5175

**Size:** 100T/96S

**Components:**

**Reagent I:** 25 mL×1. Store at 2-8°C.

**Reagent II:** 6 mL×1. Store at 2-8°C.

**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°C for 5 minutes. Measure the absorbance at 450 nm, record as A1 <sub>T</sub> , A1 <sub>B</sub> ;		
Reagent II	48	48
Mix well. React at 37°C for 5 minutes. Measure the absorbance at 450 nm, record as A2 <sub>T</sub> , A2 <sub>B</sub> . 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

$$\text{DBIL content } (\mu\text{mol/L}) = 1495.7 \times (\Delta A_T - \Delta A_B) - 15.343$$

#### B. Micro glass cuvette

$$\text{DBIL content } (\mu\text{mol/L}) = 1099.7 \times (\Delta A_T - \Delta A_B) - 10.73$$

#### Note:

1. Bilirubin decomposes easily in light. Avoid light during measurement.
2. If the  $\Delta A$  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  $\Delta A_T = A_{1T} - A_{2T} = 0.217 - 0.170 = 0.047$ ,  $\Delta A_B = A_{1B} - A_{2B} = 0.044 - 0.044 = 0$ . The calculated content is as follows:  
DBIL content ( $\mu\text{mol/L}$ ) =  $1495.7 \times (\Delta A_T - \Delta A_B) - 15.343 = 54.95 \mu\text{mol/L}$ .

#### Related products

BC5180/BC5185 Total bilirubin (TBIL) Content Assay Kit