Ver.A261101

Total Bilirubin Assay Kit

ORG285 (100Tests)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Bilirubin is formed by the breakdown of RBC's in the spleen, liver & bone marrow. Small amount of bilirubin circulates in the plasma loosely bound to albumin, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated with glucuronic acid, which forms a soluble compound. This is referred as direct bilirubin. Elevated levels are found in Hepatitis, Cirrhosis, Haemolytic jaundice, obstruction of biliary tract & drug induced reactions.

Principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Total Bilirubin reacts with diazotized sulfanilic acid in the presence of DMSO to form azobilirubin.

Kit components

Reagent	Volume	Storage
Extraction Reagent	110mL	2-8°C
Total Bilirubin Reagent	4 × 50mL	2-8°C
Total Bilirubin Activator	2 × 4mL	2-8°C
Bilirubin Artificial Standard (Concentration 10mg/dL)	4mL	2-8°C

Open Vial Stability

Once opened, the reagent is stable up to 20 days, if contamination is avoided

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat bottom plate and distilled water.

Reagent Deterioration

Turbidity or precipitation on in any kit component indicates deterioration and the component must be discarded. Values outside the recommended acceptable range for the Qualicheck Norm & Path control may also be an indication of reagent instability and associated results are invalid. Sample should be retested using a fresh vial of reagent.

Reagent Preparation

Total Bilirubin Reagent, Activator and Standard are ready to use.

Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

Operation Procedures

Sample Preparation

1. **Bacteria or cells**
   Harvest the cells and wash twice with PBS. Ideal to use 5 million cells for the assay. Add 1mL Extraction Reagent to 5 million cells and ultrasonicate (200W, work time 3 second / interval 10 second repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

   Note: Ideal proportion of Cells/Bacteria to Extraction Reagent is 1:5-10.
2. **Tissue**
   Prepare 10% tissue homogenate by adding 1mL Extraction Reagent to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant.
3. **Serum or Plasma**
   Directly use for the assay.

Unit Conversion

Traditional Unit	SI Unit	Conversion from Traditional to SI
mg/dL	µmol/L	× 17.1

Procedure Notes

	Blank	Standard	Sample
Reagent	1000µL	1000µL	1000µL
Standard	-	10µL	-
Sample	-	-	10µL
Mix and incubate for 5 minutes at room temperature. Measure the change in absorbance of standard and sample against reagent blank at 546nm.			

Calculation

With Factor :

Total Bilirubin = OD Test – OD Sample Blank × Factor

With Artificial Standard :

$$\text{Bilirubin Concentration} = \frac{\text{OD of Test} - \text{OD of Sample Blank}}{\text{OD of Standard}} \times 10$$

Note: Bilirubin standard to be read directly against DI water

Performance

Linearity

This reagent is linear up to 20mg/dL.

If the concentration is greater than linearity (20mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

Sensitivity

Lower detection Limit is 0.05mg/dL.