# Bilirubin Assay Kit

**Catalog No.:** abx298911

**Size:** 96 tests

**Detection Range:** 0.7 µmol/L - 50 µmol/L

**Sensitivity:** 0.7 µmol/L

**Storage:** Store all components at 2-8°C.

**Application:** For detection and quantification of Total Bilirubin (TBIL) concentration in serum samples.

**Introduction**

Bilirubin is a compound that is produced during the breakdown of heme in vertebrates. It is a pigment that causes the yellow color of bruises and in jaundice. Elevated levels of bilirubin can indicate excessive hemoglobin breakdown or problems with bilirubin excretion.

Abbexa's Bilirubin Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Bilirubin concentration. Under acidic conditions, insoluble indirect and direct bilirubin react with azo reagent to form azo bilirubin which has absorption maxima at 565 nm. The absorbance should be measured at 565 nm. The Total bilirubin concentration can be obtained and calculated by measuring the change of absorbance.

**Kit components**

1. 96-well microplate
2. Acid reagent: 30 ml
3. Diazonium salt: 10 ml
4. Stop solution: 5 ml
5. Standard: 2 vials
6. Plate sealer: 2

**Materials Required But Not Provided**

1. Microplate reader (565 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Incubator
8. Centrifuge
9. Vortex mixer

## Protocol

### A. Preparation of samples and reagents

#### 1. Sample

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

| Sample Type | Dilution Factor |
|---|---|
| Human Serum | 1 |
| Mouse Serum | 1 |
| Rat Serum | 1 |
| Rabbit Serum | 1 |
| Chicken Serum | 1 |
| Porcine Serum | 1 |



**Note:**

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Serum samples should not be hemolytic, and free of visible pollution.

#### 2. Reagents

Bring all the reagents to room temperature prior to use.

- **Diazonium working solution:** Dilute the Diazonium salt 1:1.2 with Acid reagent (for example, add 1 ml of Diazonium salt to 1.2 ml of Acid reagent and mix fully). Prepare immediately before carrying out the assay.
- **25 µmol/L standard solution**: Dissolve a vial standard with 2 ml of double distilled water. Prepare immediately before carrying out the assay and mix fully. Keep on ice in the dark and assay immediately.

### B. Assay Procedure

1. Set Standard, Standard control, Sample and Sample control tubes.
2. Add 80 µl of Acid reagent to all tubes.
3. Add 160 µl of Diazonium working solution to the Standard and Sample tubes.
4. Add 160 µl of double distilled water to the Standard control and Sample control tubes.
5. Add 30 µl of the prepared standard to the Standard and Standard control tubes.
6. Add 30 µl of sample to Sample and Sample control tubes.
7. Mix fully and incubate at 37°C for 5 minutes.
8. Add 20 µl of stop solution to all tubes.
9. Mix fully and incubate at 37°C for 5 minutes.
10. Set Standard, Standard control, Sample and Sample control wells on microplate.
11. Take 250 µl of reaction solution to corresponding wells and measure the OD of each well with a microplate reader at 565 nm.

### C. Calculation of Results

**Total bilirubin concentration in Serum samples:**

$$\mathrm{TBIL\ (\mu mol/L\ )} = \frac{A_2}{A_1} \times C \times f$$

where:

| | |
|---|---|
| $A_2$ | OD value of the sample $(OD_{Sample} - OD_{Sample\ control})$ |
| $A_1$ | OD value of the sample $(OD_{Standard} - OD_{Standard\ control})$ |
| $f$ | dilution factor of the sample before carrying out the assay |
| $C$ | concentration of standard (25 µmol/L) |