

## IDTox™ Total Bilirubin Colorimetric Assay Kit

SUP6005

Colorimetric Immunoassay for the determination of the Bilirubin in serum samples.

### PRODUCT DESCRIPTION

Bilirubin is a metabolite (breakdown product) of hemoglobin present in the bloodstream and liver of all mammals. In the liver, bilirubin is converted into a water soluble form (known as “direct bilirubin”). The determination of serum bilirubin is an important marker for the diagnosis of several diseases; elevated levels of bilirubin are strongly associated with hemolytic, blockage of the biliary tract, and liver disease.

The ID Labs Total Bilirubin Assay Kit is a plate-based colorimetric method for the determination of bilirubin in serum samples. This kit uses a simple and direct spectrophotometric assay to detect bilirubin directly from serum samples, enabling researchers to detect bilirubin levels in animal serum. The kit uses an aqueous diazotized sulfanilic acid reagent to modify bilirubin in the sample to an azobilirubin form which absorbs light at 550 nm. The absorption measured at 550 nm, is proportional to the concentration of total bilirubin in the sample. The kit contains sufficient materials to test 42 serum samples in duplicate. The kit also contains a control solution containing a standard (equivalent to 20 mg/dl bilirubin) which can be used to calibrate the assay and verify kit performance.

The kit uses a spectrophotometric, kinetic assay to detect changes in bilirubin levels directly from serum samples. The features of the kit are:

- High sensitivity and low detection limit (1 mg/dl)
- A rapid (5 minutes) and robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility

### PROCEDURE OVERVIEW

After preparing the sera, the assay is performed by adding serum samples into microplate wells containing sulfanilic acid and sodium nitrite reagents. The absorbance of each sample is measured again to determine the total bilirubin in the sera.

### KIT REAGENTS SUPPLIED

The Bilirubin Enzymatic Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). The kit also contains enough material to construct four standard curves. Store the kit at 4°C. The shelf life of the kit is noted on the label, when properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	RT or 2-8°C
Reagent Mix	26 ml	2-8°C
Nitrite Reagent	1.0 ml	2-8°C
Calibration Standard (20 mg/dl)	0.2 ml	2-8°C

### MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader (550 nm).
- MicroCentrifuge to prepare serum samples.
- Deionized or distilled water.
- 1.5 ml microfuge tubes
- Multichannel pipette or repeating pipettor (*recommended but not required*).
- PBS (phosphate buffer saline, pH 7.3)

### SENSITIVITY (Serum Detection Limit)

1 (mg/dl)

**For *in vitro* research use. CAUTION: Not for human or animal therapeutic use.**  
**Uses other than the labeled intended use may be a violation of local law.**

**WARNINGS AND PRECAUTIONS**

It is strongly recommended that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit.

Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic. Use only distilled or deionized water since water quality is very important. Try to maintain a laboratory temperature of (20–25°C/68–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should also be avoided.

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**ASSAY PROCEDURE**

**PREPARATION OF REAGENTS**

**Serum**

1. Carefully collect whole blood in a 1.5 ml microfuge tube or serum collection tube making sure to avoid hemolysis.
2. Incubate the blood sample at 37°C for 10 minutes.
3. Centrifuge sample at 10,000 rpm for 10 minutes.
4. Remove serum layer to a clean tube avoiding the “buffy coat” layer.
5. Store serum samples on ice or at 4°C prior to testing; do not freeze samples. Serum samples can be stored at 4°C for up to one week.

**ASSAY PROCEDURE**

**Set up**

1. Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 550 nm.
2. Allow reagents to warm up to room temperature for 30 minutes.

**Preparation of Standard Dilutions for Standard Curve**

1. Label 6 microfuge tubes: 1, 2, 3, 4, 5, 6 (Neg).
2. Dilute the Calibration Standard using distilled or deionized water as described in the table below. After dilution, briefly mix each tube before performing the next dilution.

Tube #	Calibration Standard (20 mg/dl)	Water	Concentration
1	100 µl	0 µl	20 mg/dl
2	80 µl	20 µl	16 mg/dl
3	60 µl	40 µl	12 mg/dl
4	40 µl	60 µl	8 mg/dl
5	20 µl	80 µl	4 mg/dl
6 (Neg)	0 µl	100 µl	0 mg/dl

**Assay Protocol**

1. Add 263 µl of Reagent Mix and 7 µl Nitrite Mix to the microplate wells.

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2. Add 30  $\mu$ l of serum or diluted standard to each well. Mix gently. Incubate one minute at room temperature.
3. Incubate 5 minutes at room temperature.
4. Measure the absorbance of each sample at 550 nm to determine the Total bilirubin levels.

## CALCULATION OF RESULTS

### Standard Curve Construction

A standard curve can be constructed using the serially-diluted standards by plotting the average absorbance for each standard against its concentration in mg/dl.

### Determination of Bilirubin in Serum Samples

For the Total bilirubin standard curve, calculate the slope and the y-intercept for the line which best fits the standard curve data plot.

The Total bilirubin concentration in each sample can be described by the equation:

Total bilirubin concentration = (mean absorbance – y-intercept)/slope

Use the mean absorbance values for each serum sample to determine the corresponding concentration of bilirubin from the standard curve.

Note: Samples with values above 20 mg/dl should be diluted 1:1 with PBS and re-tested. Multiply results by 2.

