

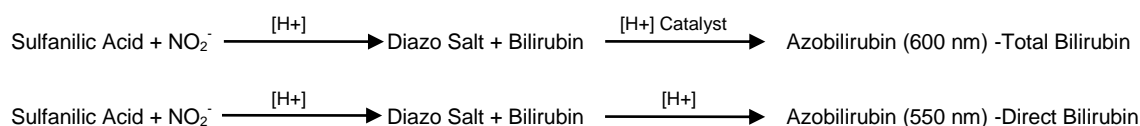
# Bilirubin (Total and Direct) Colorimetric Assay Kit

(Catalog # K553-100; 100 assays; Store at -20°C)

10/14

## I. Introduction:

Bilirubin, a degradation product of heme catabolism, is a non-polar molecule. There are two forms of bilirubin: water-soluble (conjugated or direct) and water-insoluble (unconjugated or indirect) bilirubin. Bilirubin is produced in the endoplasmic reticulum as unconjugated bilirubin, which binds to albumin in plasma and forms albumin-bilirubin complex. This complex is transported to the liver, where it is conjugated with glucuronic acid and forms conjugated bilirubin. Bilirubin has potent antioxidant, anti-inflammatory and autoimmune properties. Bilirubin concentration in human body depends on gender, drug intake, age, etc. Low serum bilirubin is directly correlated with pathological conditions including diabetes mellitus, metabolic syndrome, and cardiovascular diseases. However, high bilirubin indicates hemolysis, jaundice, Gilbert's syndrome, hepatitis, drug toxicity, and possible blockage of bile ducts. BioVision's Bilirubin Assay Kit utilizes the Jendrassik-Grof principle to detect bilirubin. Total bilirubin (unconjugated + conjugated) concentration is determined in the presence of a catalyst, where bilirubin reacts with a diazo- salt to form azobilirubin, which absorbs at 600 nm. Direct bilirubin (conjugated) is determined in the absence of catalyst (550 nm).



## II. Application:

- Measurement of bilirubin concentration in serum

## III. Sample Type:

- Biological fluids: Serum

## IV. Kit Contents:

| Components                     | K553-100   | Cap Code | Part Number |
|--------------------------------|------------|----------|-------------|
| Bilirubin Reagent 1            | 2.5 ml     | NM       | K553-100-1  |
| Bilirubin Reagent 2            | 1 ml       | Red      | K553-100-2  |
| Catalyst                       | 15 ml      | NM       | K553-100-3  |
| Total Bilirubin Probe          | 10 ml      | NM       | K553-100-4  |
| Direct Bilirubin Probe         | 20 ml      | WM       | K553-100-5  |
| Bilirubin Standard (0.2 µg/µl) | 2 x 200 µl | Yellow   | K553-100-6  |
| DMSO (Anhydrous)               | 3.5 ml     | NM       | K553-100-7  |

## V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer or plate reader

## VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay. Once opened, all kit components can be stored and used for up to 6 months.

- **Bilirubin Reagent 1:** Bring to room temperature (RT) before use. Store at -20°C.
- **Bilirubin Reagent 2:** Ready to use as supplied. Light sensitive. Warm to RT before use. Store at -20°C.
- **Catalyst:** Ready to use as supplied. Bring to RT before use. Store at -20°C.
- **Total Bilirubin Probe:** Bring to RT before use. Store at -20°C.
- **Direct Bilirubin Probe:** Ready to use as supplied. Warm to RT before use. Store at -20°C.
- **Bilirubin Standard:** Ready to use as supplied. Bring to RT before use. **Light and Oxygen sensitive. Aliquot into amber vials and store at -20°C.**
- **DMSO:** Warm to RT to liquefy completely before use.

## VII. Bilirubin Assay Protocol:

1. **Sample Preparation:** Add 2-50 µl of undiluted serum to desired well(s) in a 96-well plate. Adjust the volume to 50 µl/well with 50 % DMSO (mix 500 µl 100% DMSO (provided) and 500 µl ddH<sub>2</sub>O for about 20 wells).

### Note:

Bilirubin concentration varies over a wide range depending on the patient's age, gender, and pathological conditions. In healthy patients, bilirubin concentrations (in mg/dl) are: Total: (0.1- 1.2); Indirect: (0.1- 0.7); Direct: (0.1- 0.4). For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

**2. Standard Curves Preparation:** Dilute 50  $\mu\text{l}$  of 0.2  $\mu\text{g}/\mu\text{l}$  Bilirubin standard with 50  $\mu\text{l}$  100% DMSO (provided). Add 0, 2.5, 5, 10, 20, 40  $\mu\text{l}$  of the diluted Bilirubin standard (0.10  $\mu\text{g}/\mu\text{l}$ ) into a series of wells in a 96-well plate to generate 0, 0.25, 0.5, 1, 2, and 4  $\mu\text{g}$  of Bilirubin/well. Adjust the final volume to 50  $\mu\text{l}$ /well with 50% DMSO.

**3. Total Bilirubin Reagent Mix:** For Total Bilirubin determination, mix enough reagents for the total number of well(s) to be assayed including Standards, Samples, and Background Controls. Add 125  $\mu\text{l}$  of Reagent Mix to each well. Mix well.

|                     | Standard/Sample   | Background Control |
|---------------------|-------------------|--------------------|
| Bilirubin Reagent 1 | 20 $\mu\text{l}$  | 20 $\mu\text{l}$   |
| Bilirubin Reagent 2 | 5 $\mu\text{l}$   | —                  |
| ddH <sub>2</sub> O  | —                 | 5 $\mu\text{l}$    |
| Catalyst            | 100 $\mu\text{l}$ | 100 $\mu\text{l}$  |

Mix well. Incubate plate for 30 min at RT (~25°C). Add 75  $\mu\text{l}$  of Total Bilirubin Probe to all Standards, Samples and Background Control. Incubate plate at RT for 20 min., protected from light. Measure end point absorbance at 600 nm in a plate reader.

**4. Direct Bilirubin Reagent Mix:** For Direct Bilirubin determination, mix enough reagents for the total number of well(s) to be assayed including Standard, Samples and Background Controls. Add 25  $\mu\text{l}$  of Reagent Mix to each well. Mix well.

|                     | Standard/Sample  | Background Control |
|---------------------|------------------|--------------------|
| Bilirubin Reagent 1 | 20 $\mu\text{l}$ | 20 $\mu\text{l}$   |
| Bilirubin Reagent 2 | 5 $\mu\text{l}$  | —                  |
| ddH <sub>2</sub> O  | —                | 5 $\mu\text{l}$    |

Mix well. Incubate plate for 30 min at 25°C. Add 175  $\mu\text{l}$  of Direct Bilirubin Probe to all Standards, Samples and Background Control. Incubate plate at RT for 20 min., protected from light. Measure end point absorbance at 550 nm in a plate reader.

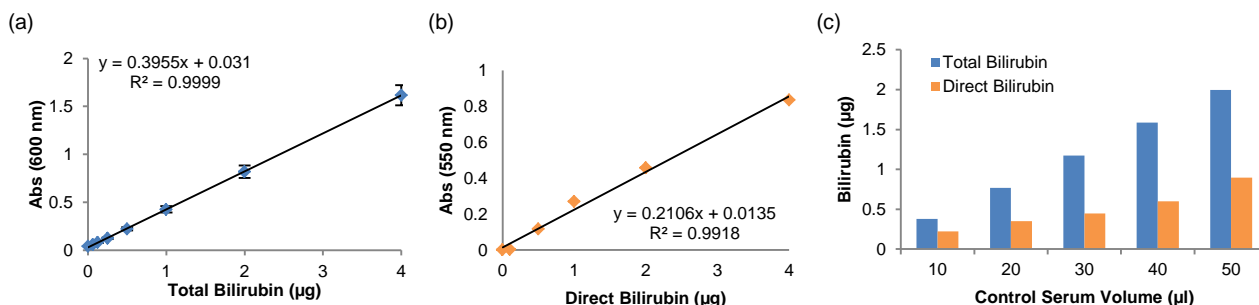
**5. Calculation:** Subtract 0 Total Bilirubin Standard reading from all standard readings. Plot the Linear Total Bilirubin Standard Curve. If sample background control is significant (i.e. if sample background control for Total Bilirubin/Direct Bilirubin has intrinsic high absorbance at 600/550 nm), then subtract sample background control reading from sample readings. Apply the corrected OD to the Total Bilirubin Standard Curve to get B  $\mu\text{g}$  of Total Bilirubin in the sample well. Fit the Direct Bilirubin Standard values to a polynomial trend-line and solve the quadratic equation of the polynomial Standard curve (Fig. b) to obtain B  $\mu\text{g}$  of Direct Bilirubin in the sample well.

$$\text{Sample Total or Direct Bilirubin Concentration (C)} = \text{B/V} \times \text{D} \mu\text{g}/\mu\text{l}$$

Where: **B** is the amount of Total/Direct Bilirubin in the sample well ( $\mu\text{g}$ )  
**V** is the sample volume added into the reaction well ( $\mu\text{l}$ )  
**D** is the sample dilution factor

**Total Bilirubin = Unconjugated Bilirubin + Conjugated Bilirubin**

Bilirubin Molecular Weight: 584.7 kDa  
 10 mg/ml  $\equiv$  10  $\mu\text{g}/\mu\text{l}$   $\equiv$  10000  $\mu\text{g}/\text{ml}$   $\equiv$  1 g/dl



**Figure:** (a) Total Bilirubin Standard Curve (0 - 4  $\mu\text{g}$ ). (b) Direct Bilirubin Standard Curve (0 - 4  $\mu\text{g}$ ). Total and Direct Bilirubin concentrations in Normal Human Serum. Different volumes of human serum (10- 50  $\mu\text{l}$ ) were assayed following kit protocols. Reported concentrations (in mg/dl): Total Bilirubin: 4; Direct Bilirubin: 1.5. Experimental concentrations (calculated as the average of estimated bilirubin in five different human serum volumes ranging from 10 to 50  $\mu\text{l}$ ): Total: 3.8 mg/dl; Direct: 1.75 mg/dl

## VII. Related Products:

Albumin (BCG) Colorimetric Assay Kit (K554)  
 Human Serum Albumin (4016)  
 Albumin, Human plasma (7546)

**FOR RESEARCH USE ONLY! Not to be used on humans.**