**DESCRIPTION**

**BILIRUBIN** is one of the degradation products of hemoglobin formed when red blood cells die. Bilirubin exists in the insoluble unconjugated form (also indirect bilirubin), or soluble glucuronide conjugated form (direct bilirubin). Conjugated bilirubin moves into the bile canaliculi of the liver and then to the gall bladder. When stimulated by eating, bile (including the conjugated bilirubin) is excreted into the small intestine, where bilirubin is converted into urobilinogen. Bilirubin is a key diagnostic indicator. High levels of bilirubin result when too much hemoglobin is broken down or the removal of bilirubin does not function properly. The accumulation of bilirubin in the body causes jaundice.

Simple and automation-ready procedures for quantitative determination of bilirubin find wide applications in research and drug discovery. BioAssay Systems’ bilirubin assay kit is designed to measure bilirubin in blood specimen in 96-well or cuvette formats. The improved Jendrassik-Grof method utilizes the reaction of bilirubin with diazotized sulfanilic acid, in which a red colored product is formed. The intensity of the color, measured at 510-550nm, is an accurate measure of the bilirubin level in the sample. Total bilirubin is assessed using caffeine benzoate to split bilirubin from the unconjugated bilirubin protein complex.

**KEY FEATURES**

- **Sensitive and accurate.** Detection limit 0.16 mg/dL bilirubin in 96-well plate assay.
- **Simple and high-throughput.** The procedure involves addition of a single working reagent and incubation for 10 min. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.

**APPLICATIONS:**

- **Direct Assays:** total and direct bilirubin in serum.
- **Pharmacology:** effects of drugs on bilirubin metabolism.

**KIT CONTENTS (180 tests in 96-well plates)**

- **Reagent A:** 30 mL
- **Reagent B:** 10 mL
- **Reagent C:** 30 mL
- **Saline:** 50 mL
- **Calibrator:** 2 mL (equivalent to 5 mg/dL Bilirubin).

**Storage conditions.** The kit is shipped at room temperature. Store all reagents at 4°C. Shelf life: 12 months after receipt.

**Precautions.** Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

**PROCEDURES**

Hemolysis interferes with the assay. Avoid exposure of sample to any light. Samples can be stored at −20°C for up to 3 months, 2–8°C for 4 days. Note: Some samples, especially those with high lipid content, may cause turbidity when mixing with the reagents. If turbidity is observed, centrifuge and use clear supernatant for assay.

**Procedure using 96-well plate:**

1. **Reagent Preparation:** prepare at least 200 µL/well fresh Working Reagent as follows.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Saline</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>50 µL</td>
<td>20 µL</td>
<td>130 µL</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Direct</td>
<td>50 µL</td>
<td>20 µL</td>
<td>–</td>
<td>130 µL</td>
<td>–</td>
</tr>
<tr>
<td>Blank</td>
<td>50 µL</td>
<td>–</td>
<td>–</td>
<td>130 µL</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

*Total Bilirubin* is determined with Working Reagent that contains Reagent C, and *Direct Bilirubin* with Working Reagent that does not contain Reagent C but saline instead.

2. **Calibrator:** transfer 50 µL H₂O and 50 µL Calibrator into two wells of clear-bottom 96-well plate, add 200 µL H₂O. The volume in each well 250 µL.

3. **Incubate 10 min and read** OD530 nm (510 to 550 nm).

**CALCULATION**

\[
\text{Bilirubin} = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{CALIBRATOR}} - \text{OD}_{\text{H₂O}}} \times 5 \text{ (mg/dL)}
\]

where \(\text{OD}_{\text{SAMPLE}}, \text{OD}_{\text{BLANK}}, \text{OD}_{\text{CALIBRATOR}}\) and \(\text{OD}_{\text{H₂O}}\) are the OD₅₃₀nm values of the sample, the sample blank, the calibrator and water. 5 (mg/dL) is the equivalent bilirubin concentration of the calibrator.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

- Pipeting devices and accessories, 96-well plates and plate reader.

**PUBLICATIONS**

