



Bilirubin Assay Kit (Colorimetric)

Bilirubin Assay Kit (Colorimetric) is a detection kit for the quantification of Bilirubin in serum.

Catalog number: ARG82141

Package: 180 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Bilirubin (BR) is a yellow compound that occurs in the normal catabolic pathway that breaks down heme in vertebrates. This catabolism is a necessary process in the body's clearance of waste products that arise from the destruction of aged or abnormal red blood cells. First the hemoglobin gets stripped of the heme molecule which thereafter passes through various processes of porphyrin catabolism, depending on the part of the body in which the breakdown occurs. For example, the molecules excreted in the urine differ from those in the feces. The production of biliverdin from heme is the first major step in the catabolic pathway, after which the enzyme biliverdin reductase performs the second step, producing bilirubin from biliverdin.

Ultimately, the metabolites of bilirubin are excreted through bile and urine, and elevated levels may indicate certain diseases. It is responsible for the yellow color of bruises and the yellow discoloration in jaundice. Its breakdown products, such as stercobilin, cause the brown color of feces. A different breakdown product, urobilin, is the main component of the straw-yellow color in urine. [Provide by Wikipedia: Bilirubin]

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PRINCIPLE OF THE ASSAY

This Bilirubin Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of bilirubin present in serum. The Bilirubin Assay kit 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 O.D. 530 nm, 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.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store all components at 4°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Reagent A	30 mL	4°C
Reagent B	10 mL	4°C
Reagent C	30 mL	4°C
Salin	50 mL	4°C
Standard (5 mg/dL Bilirubin)	2 mL	4°C

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MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 510-550 nm
- Centrifuge
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Hemolysis interferes with the assay. Avoid exposure of sample to any light.
- 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.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

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SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Note:

1. Hemolysis interferes with the assay. Avoid exposure of sample to any light.
2. 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.
3. Samples can be store at -20°C for up to 3 months, 2-8°C for 4 days.

REAGENT PREPARATION

- **Working Reagent:** prepare at least 200 µL/well fresh Working Reagent as follows,

	Reagent A	Reagent B	Reagent C	Saline	Distilled water
Total	50 µL	20 µL	130 µL		
Direct	50 µL	20 µL		130 µL	
Blank	50 µL			130 µL	20 µL

Note:

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

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ASSAY PROCEDURE

Equilibrate reagents to desired reaction temperature (E.g., 25°C or 37°C). Briefly centrifuge tubes before use.

1. Add **50 µL** of **distilled water** and **50 µL** of **Standard** into two wells of clear bottom 96-well microplate, and then add **200 µL** of **distilled water**. **The volume in each well is 250 µL.**
2. Add **50 µL** of **sample** into separate wells, add **200 µL** of respective **Working Reagent** (E.g., for **total bilirubin** or **direct bilirubin**) and **200 µL** of **Blank reagent** to the sample well.
3. Incubate for **10 minutes** and read the absorbance at **O.D. 530 nm (510-550 nm)**

CALCULATION OF RESULTS

1. $\text{Bilirubin (mg/dL)} = \frac{(\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}})}{(\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{DISTILLED WATER}})} \times 5$

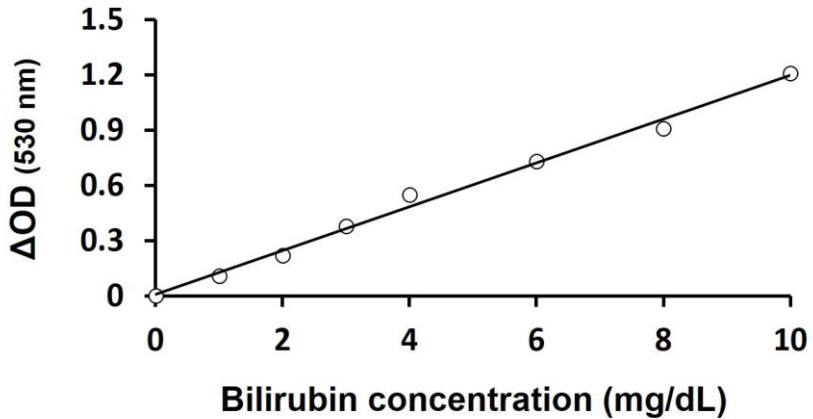
Note:

- $\text{OD}_{\text{SAMPLE}}$, OD_{BLANK} , $\text{OD}_{\text{STANDARD}}$ and $\text{OD}_{\text{DISTILLED WATER}}$: the O.D. 530 nm values of the sample, sample blank, Standard and distilled water.
- 5: the equivalent bilirubin concentration of the calibrator.

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EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Bilirubin Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.16 mg/dL