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User's Manual and Instructions

Bilirubin Assay Kit (Z5030053)

Quantitative Colorimetric Bilirubin Determination at 530nm

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 bilirubin (also 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. Biochain's 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 or plasma.
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 store at –20°C for up to 3 months, 2–8°C for 4 days. If turbidity is observed, centrifuge sample 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,

	A	B	C	Saline	H_2O
Total	50 µL	20 µL	130 µL	–	–
Direct	50 µL	20 µL	–	130 µL	–
Blank	50 µL	–	–	130 µL	20 µL

"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 H_2O and 50 µL Calibrator into two wells of clear-bottom 96-well plate, add 200 µL H_2O. The volume in each well 250 µL.

   Samples: transfer 50 µL sample into separate wells, add 200 µL respective Working Reagent (i.e. for total bilirubin and/or direct bilirubin) and 200 µL "Blank" Reagent to the sample wells.

3. Incubate 10 min and read OD530nm (510 to 550nm).

Procedure using Cuvet:

1. Prepare at least 800 µL/well fresh Working Reagent as follows,

	A	B	C	Saline	H_2O
Total	200 µL	80 µL	520 µL	–	–
Direct	200 µL	80 µL	–	520 µL	–
Blank	200 µL	–	–	520 µL	80 µL

2. Transfer 200 µL H_2O and 200 µL Calibrator into two wells of clear-bottom 96-well plate, add 800 µL H_2O. Transfer 200 µL sample, add 800 µL Working Reagent.
3. Incubate 10 min and read OD530nm (510 to 550nm).

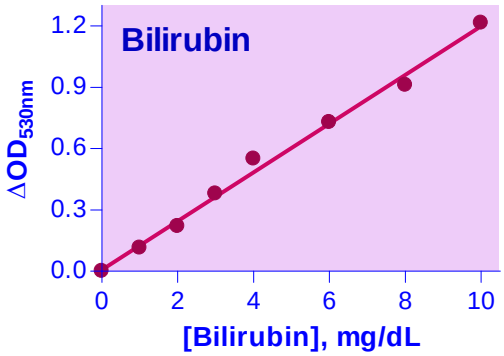
CALCULATION

$$\textbf{Bilirubin} = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{CALIBRATOR} - OD_{H2O}} \times 5 \text{ (mg/dL)}$$

where OD_{SAMPLE}, OD_{BLANK}, $OD_{CALIBRATOR}$ and OD_{H2O} are the OD_{530nm} 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.



Standard Curve with Freshly Prepared Bilirubin in 5g/dL Bovine Serum Albumin in 96-well plate assay

PUBLICATIONS

[1]. Vinchi F et al. 2008. Hemopexin prevents endothelial damage and liver congestion in a mouse model of heme overload Am J Pathol. 173(1): 289–299

[2]. Nedredal GI et al. 2009. Optimization of mass transfer for toxin removal and immunoprotection of hepatocytes in a bioartificial liver. Biotechnol Bioeng. 104(5):995-1003.

[3]. Beppu F. et al. 2009. Single and repeated oral dose toxicity study of fucoxanthin (FX), a marine carotenoid, in mice. J. Toxicol. Sci. 34(5): 501-510.