Product Manual

Bilirubin Assay Kit

Catalog Number

MET-5010 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Bilirubin is an open chain molecule containing four pyrrole-like rings that forms during the breakdown of heme. Bilirubin is excreted in urine and bile and can also be found in low levels in plasma. Three principal forms of bilirubin are found in plasma: conjugated (to glucuronic acid; also called direct bilirubin which makes bilirubin water soluble), unconjugated, or bound to serum albumin. Eventually, bilirubin is degraded in the liver to be removed from the body. While high levels of bilirubin in serum have been correlated with jaundice, hepatitis, Gilbert's syndrome, and drug toxicity, low levels of bilirubin have been correlated with cardiovascular disease, diabetes mellitus, and metabolic syndrome.

Cell Biolabs' Bilirubin Assay Kit is a simple colorimetric assay that measures the amount of total bilirubin present in plasma, serum, cell lysates, or tissue lysates in a 96-well microtiter plate format. The kit has a detection sensitivity limit of 0.5 mg/dL bilirubin. Each kit provides sufficient reagents to perform up to 200 assays*, including blanks, bilirubin standards and unknown samples. Sample bilirubin concentrations are determined by comparison with a known bilirubin standard.

Note: Each sample replicate requires 2 assays, one treated with a Diazo Reagent and one without (negative control). The Net OD is calculated from the difference in OD readings from the two wells.

Assay Principle

Cell Biolabs' Bilirubin Assay Kit measures the total bilirubin within serum, plasma, cell lysates, or tissue lysate samples. The assay is based on the Jendrassik-Grof method (Ref. 1) in which diazotized sulfanilic acid reacts with bilirubin to form azobilirubin, the latter of which can be detected at an OD of 540 nm. Since unconjugated bilirubin and bilirubin bound to albumin react very slowly, an accelerant is added to the reaction to allow for measurement of total bilirubin.

Diazotized Sulfanilic Acid + Total Bilirubin + Accelerant → Azobilirubin (540 nm)

Related Products

1. STA-378: Urinary Creatinine Assay Kit
2. STA-631: Total Bile Acid Assay Kit (Coloimetric)
3. MET-5071: Taurine Assay Kit
4. STA-620: Alcohol Assay Kit (Colorimetric)
5. MET-5012: Lactate Assay Kit (Colorimetric)

Kit Components

1. Bilirubin Standard (Part No. 50101C): One 240 µL vial of an 800 mg/dL solution in DMSO.
2. Accelerant (Part No. 50102C): One 30 mL bottle.
3. Diazo Reagent (Part No. 50103C): One 6 mL bottle.
4. Negative Control Reagent (Part No. 50104C): One 6 mL bottle.
5. Assay Reagent A (Part No. 50105C): One 15 mL bottle.
6. Assay Reagent B (Part No. 50106C): One 1.5 mL vial.

Materials Not Supplied

1. 96 well plate or 96 well strips
2. Deionized water

3. 1X PBS

Storage
Store the kit at -20ºC.

Preparation of Reagents
- Accelerant, Diazo Reagent, and Negative Control Reagent: Warm at 37°C for 5-10 minutes until completely thawed. Vortex on high speed for 30 seconds or until completely resuspended.

Preparation of Samples
Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Aliquot the supernatant for storage at -80ºC. Perform dilutions in deionized H_2O.
- Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Aliquot the supernatant for storage at -80ºC. Perform dilutions in deionized H_2O.
- Serum: Avoid hemolyzed and lipemic blood samples. 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. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in deionized H_2O as necessary.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in deionized H_2O as necessary.

Preparation of Standard Curve
Prepare fresh Bilirubin standards by diluting in deionized H_2O according to Table 1.

Tubes	800 mg/dL Bilirubin Standard (µL)	Deionized H_2O (µL)	Resulting Bilirubin Concentration (mg/dL)
1	12	288	32
2	150 of Tube #1	150	16
3	150 of Tube #2	150	8
4	150 of Tube #3	150	4
5	150 of Tube #4	150	2
6	150 of Tube #5	150	1
7	150 of Tube #6	150	0.5
8	0	150	0

Table 1. Preparation of Bilirubin Standards.

Assay Protocol

Each Bilirubin standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed. For each reaction using Diazo Reagent, a second well containing Negative Control Reagent instead of Diazo Reagent should be performed to determine background (see step 3 below).

1. Add 50 µL of the diluted Bilirubin standards or samples to the 96-well microtiter plate.
2. Add 125 µL of Accelerant to each well and mix the well contents thoroughly.
3. Add 25 µL of Diazo Reagent (or 25 µL of Negative Control Reagent to negative control wells)
4. Add 75 µL of Assay Reagent A to each well.
5. Incubate at room temperature for 1 hour protected from light.
6. Add 5 µL of Assay Reagent B to each well.
7. Read the plate at wavelength of 540 nm using a 96-well plate spectrophotometer.
8. Calculate Net OD for each sample by subtracting OD from negative control wells from each sample well.

Example of Results

The following figures demonstrate typical Bilirubin Assay results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

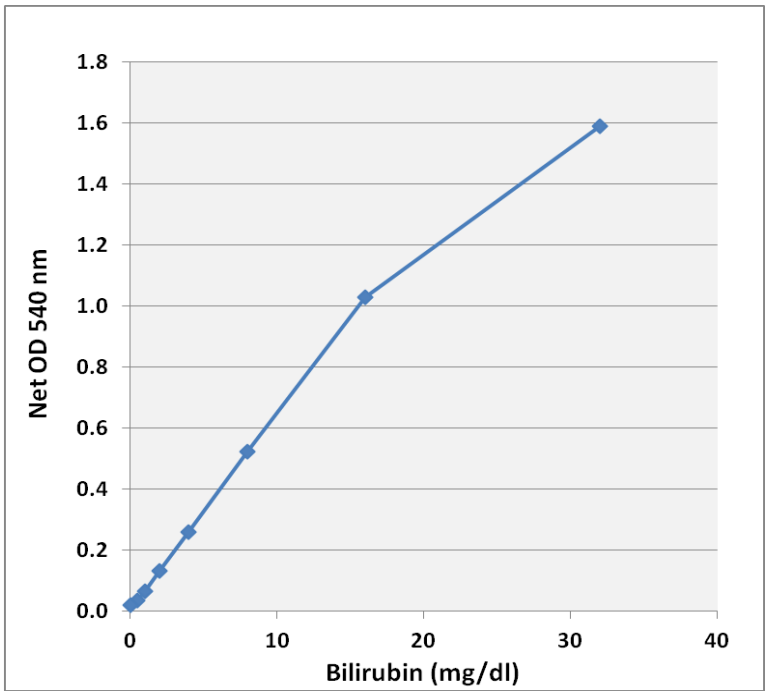

Figure 1: Total Bilirubin Standard Curve.

References

1. Jendrassik L, Grof P. (1938) *Biochem Z* **297**: 81-89.
2. Doumas BT, Perry BW, Jenderzejczak B, and Katona V. (1982) *Clin. Chem* **28**: 2305-2308.
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Recent Product Citations

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2. Choi, H.J. et al. (2019). Efficacy and safety of a novel topical agent for gallstone dissolution: 2-methoxy-6-methylpyridine. *J Transl Med*. **17**(1):195. doi: 10.1186/s12967-019-1943-y.
3. Yokoyama, T. et al. (2019). Regulation of CCl4-induced liver cirrhosis by hepatically differentiated human dental pulp stem cells. *Hum Cell*. **32**(2):125-140. doi: 10.1007/s13577-018-00234-0.
4. Thangamuthu, M. et al. (2018). Electrochemical Sensor for Bilirubin Detection Using Screen Printed Electrodes Functionalized with Carbon Nanotubes and Graphene. *Sensors (Basel)*. **18**(3). pii: E800. doi: 10.3390/s18030800.

Warranty

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