

Technical Bulletin

Bilirubin Assay Kit

Catalogue number MAK126

Product Description

Bilirubin, also known as hematoidin, is a degradation product formed as a result of heme catabolism in the liver. Bilirubin circulates in the blood stream as either the unconjugated insoluble form (indirect bilirubin) or the soluble glucuronide-conjugated form (direct bilirubin). Conjugated bilirubin moves from the bile canaliculi of the liver to the gall bladder where it is excreted into the small intestine during digestion. High levels of bilirubin can result in jaundice and may indicate liver disease, blood disorders, or blockage of the bile ducts.

The Bilirubin Assay kit provides a simple and direct procedure for measuring bilirubin in serum. This assay, based on the Jendrassik-Grof method, utilizes the reaction of bilirubin with diazotized sulfanilic acid resulting in a colorimetric product measured at 530 nm, proportionate to the bilirubin present in the sample. This assay kit measures both total and conjugated bilirubin.

Components

The kit is sufficient for 180 assays in 96 well plates.

Reagent A Catalog Number MAK126A	30 mL
Reagent B Catalog Number MAK126B	10 mL
Reagent C Catalog Number MAK126C	30 mL
Saline Catalog Number MAK126D	50 mL
Calibrator Catalog Number MAK126E	2 mL

Reagents and Equipment Required but Not Provided

- Spectrophotometric multiwell plate reader
- Clear 96 well flat-bottom plate

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped at room temperature. Storage at 2-8 °C, protected from light, is recommended.

Procedure

Sample Preparation

Hemolysis interferes with this assay. Samples should be protected from light to prevent photodegradation of bilirubin in the Sample. Samples can be stored at -20 °C for up to 3 months or 2-8 °C for 4 days. If turbidity is observed in the Samples, Samples can be centrifuged, and the cleared supernatant used for assays.

Assay Reaction for 96 well plate

1. Prepare at least 200 µL of Working Reagents (Total, Direct, and Blank) for each well as indicated in Table 1. Working Reagents should be prepared fresh for each assay. Total Bilirubin is determined by the addition of Reagent C containing caffeine benzoate which splits bilirubin from the unconjugated bilirubin-protein complex. Prepare enough of the Total, Direct, and Blank Working Reagents for each Sample tested.

Table 1.

Working Reagents for 96 well plate

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

2. Transfer 50 µL of calibrator and 50 µL of water into separate wells of a 96 well plate. Add 200 µL of water into each well for a final volume of 250 µL.
3. Transfer 50 µL of Samples into separate wells of the plate for the total, direct, and blank measurement. Transfer 200 µL of the appropriate Working Reagent to each of the Sample wells.
4. Incubate at room temperature for 10 minutes.
5. Measure the absorbance at 530 nm (A_{530}).

Assay Reaction for Cuvette

1. Prepare at least 800 µL of Working Reagents (Total, Direct, and Blank) for assay as indicated in Table 2. Prepare enough of the Total, Direct, and Blank Working Reagent for each Sample tested.

Table 2.

Working Reagents for Cuvette

	A	B	C	Saline	Water
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 of calibrator and 200 µL of water into two cuvettes. Add 800 µL of water into each cuvette for a final volume of 1,000 µL.
3. Transfer 200 µL of Sample into cuvettes (one each for Total, Direct, and Blank measurement). Transfer 800 µL of the appropriate Working Reagent to each of the cuvettes.
4. Incubate at room temperature for 10 minutes.
5. Measure the absorbance at 530 nm (A_{530}).

Calculations

Bilirubin concentration

$$= \frac{(A_{530})_{\text{sample}} - (A_{530})_{\text{blank}}}{(A_{530})_{\text{calibrator}} - (A_{530})_{\text{water}}} \times (5 \text{ mg/dL})$$

where:

$(A_{530})_{\text{sample}}$ = Value of the sample (Total or Direct)

$(A_{530})_{\text{Blank}}$ = Value of the sample Blank

$(A_{530})_{\text{calibrator}}$ = Value of the calibrator

$(A_{530})_{\text{water}}$ = Value of the water control

5 mg/dL = equivalent bilirubin concentration of the calibrator when assay performed as indicated

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples underwent hemolysis or were exposed to light	Prepare fresh samples
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings

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mak126pis Rev 11/22

