



# BILIRUBIN Total

Colorimetric determination of total Bilirubin  
in serum or plasma



## ORDER INFORMATION

REF	Kit size
GA4231 00	10x20 + 1x10 ml
KL4231 00	10x20 + 1x10 ml
BK4231 00	5x(50+5 ml)

## INDICATION

Determination of total and direct bilirubin is used for the diagnosis and monitoring of hepatic (hepatitis, cirrhosis) and hemolytic and biliary disorders.

## METHOD PRINCIPLE

In the present method (Jendrassik modified) total Bilirubin, in the presence of Diazosulphanilic acid, forms a coloured compound (Azobilirubin) which absorbs at 546 nm. The colour intensity is proportional to the total Bilirubin concentration present in the sample.

## COMPOSITION

### REAGENT A (liquid):

Sulphanilic acid 6 mmol/l  
DMSO 7 mol/l  
Surfactants and preservatives

### REAGENT B (liquid):

Sodium nitrite 20 mmol/l

## Preparation

Mix 20 part of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g. 40 ml of RA + 2 ml of RB).

## Storage and stability

Store at room temperature (15-25 °C). Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 7 days at 2-8 °C.

## ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution 9 g/l
- Calibrator (GD CAL Ref. GD8577 00)

## SAMPLES

Fresh unhemolysed serum or plasma. Serum samples must be assayed within 2 hours.

Sample turbidity due to macromolecular aggregates may interfere. In this case, centrifuge or filtration through a 0.2 µ membrane filter is suggested. Avoid light exposure.

## Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

## INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known total Bilirubin concentration. Check that the values obtained are within the reference range provided.

## ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes:

	Blank	Calibrator	Sample
Sample	-	-	100 µl
Distilled H <sub>2</sub> O	100 µl	-	-
Calibrator	-	100 µl	-
Working reagent	1000 µl	1000 µl	1000 µl

Mix and incubate for **10 minutes** at **room temperature** (20-25 °C). Read absorbance A for each cuvette at **570 (550-580) nm** against Blank cuvette.  
The colour is stable 30 minutes at room temperature.

## CALCULATION OF RESULTS

$$\text{Total Bilirubin, mg/dl} = \frac{A \text{ sample}}{A \text{ calibrator}} \times \text{mg/dl calibrator}$$

Against factor:

$$\text{Total Bilirubin, mg/dl} = A \times 13.08$$

## Note:

Use factor calculation when instrumentation is able to select a passing band not too wide. It is suggested to use standard calculation with instruments not having this condition.

## REFERENCE VALUES

0.1 ÷ 1.2 mg/dl

Each laboratory should establish reference ranges for its own patients population.

## ANALYTICAL PERFORMANCES

### Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different total Bilirubin concentrations. The obtained results are reported in the following table:

Sample	Mean (mg/dl)	Within Run		Between Run	
		SD	%CV	SD	%CV
Serum 1	1.06	0.01	0.9	0.10	9.4
Serum 2	2.28	0.01	0.4	0.11	4.8
Serum 3	3.51	0.01	0.3	0.13	3.7

### Linearity

The assay is linear up to 20 mg/dl.

### Sensitivity

Test sensitivity, in terms of limit of detection, is 0.1 mg/dl.

### Correlation

A correlation study comparing the present method and a commercial one gave the following results:

$$y = 0.9619x - 0.0326 \text{ mg/dl} \quad r = 0.9926$$

### Interferences

Hemoglobin > 500 mg/dl  
Triglycerides > 2000 mg/dl

**PRECAUTIONS IN USE**

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

**Waste Management**

Please refer to local legal requirements.

**BIBLIOGRAPHY**

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4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
5. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC