



BILIRUBIN Direct - L

Colorimetric determination of direct Bilirubin
in serum or plasma



ORDER INFORMATION

REF	Kit size
GA4256 00	10x15 + 1x10 ml
KL4256 00	10x15 + 1x10 ml
BK4256 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.

In particular, high levels of direct bilirubin (or "conjugated" bilirubin"), generally absent or present only in negligible quantities, are relieved in the following cases:

- extra hepatic biliar disorders (e.g.: gallbladder and choledochal calculi, pancreas tumor);
- inter hepatic biliar disorders (e.g.: cirrhosis, hepatitis and liver tumor).

METHOD PRINCIPLE

In the present Jendrassik modified method, total bilirubin, in the presence of diazosulphanilic acid, forms a coloured compound (azobilirubin). The colour intensity is proportional to the direct bilirubin concentration present in the sample.

COMPOSITION

REAGENT A (liquid):

Sulphanilic acid	30 mmol/l
Hydrochloric acid	0.25 mol/l

REAGENT B (liquid):

Sodium nitrite	≤ 10 mmol/l
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Preparation

Mix 15 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g. 30 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)
- Temperature controlled water bath
- NaCl solution 9 g/l
- Calibrator (GD CAL Ref. GD8577 00)

SAMPLES

Serum, sodium heparin or EDTA-Na₂ plasma. Do not use hemolyzed samples. For hyperlipemic, not limpid sample it is necessary to use sample blank. As bilirubin is a photosensitive pigment, samples must be stored protected from light and from heat. Samples must be analyzed immediately.

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 direct 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 ₂ 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.

Note:

- Reaction volumes can be proportionally changed.
- For concentration > 20 mg/dl, the sample should be diluted 1+9 with NaCl solution (0.9 g/l) and result multiplied by 10.

CALCULATION OF RESULTS

$$\text{Direct bilirubin, mg/dl} = \frac{\text{A sample}}{\text{A calibrator}} \times \text{mg/dl calibrator}$$

Conversion factor

$$\text{Direct bilirubin [mg/dl]} \times 17.1 = \text{Direct bilirubin [µmol/l]}$$

REFERENCE VALUES

Adults: up to 0.25 mg/dl (4.3 µmol/l)

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 direct 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	0.84	0.01	1.2	0.05	5.9
Serum 2	1.43	0.02	1.4	0.11	7.7
Serum 3	1.95	0.01	0.5	0.14	7.2

Linearity

The assay is linear up to 20 mg/dl (342 µmol/l).

Sensitivity

Test sensitivity, in terms of limit of detection, is 0.03 mg/dl (0.51 µmol/l).

Correlation

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

$$y = 0.9705x + 0.0778 \text{ mg/dl} \quad r = 0.9585$$

Interferences

Hemoglobin and lipids interfere with the results: hemoglobin interference may cause false low results, while lipemic samples may cause an increase in the values due to the turbidity.

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

1. Jakobs DS, Kasten Jr. BL, Demmott WR, Wolfson WL: "Laboratory Test Handbook", Lexi-Comp and Williams & Wilkins Ed. 2nd Edition (1990).
2. Chitto G, Fabi A, Franzini C, Galletta G, Leonardi A, Marelli M, Morelli AM: Variabilità biologica intra-individuo: rassegna della letteratura, contributo sperimentale e considerazioni critiche. *Biochimica Clinica*, 1994; 18, 10:673.
3. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. 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.