BILIRRUBINA DIRETA

Bilirubin Direct

Anvisa 80115310048

ORDER INFORMATION

Cat. No.	Kit size
1080075K	R1: 3 x 20mL + R2: 1 x 15 mL
1080250K	R1: 5 x 40 mL + R2: 1 x 50 mL
1080075M	R1: 3 x 20 mL + R2: 1 x 15 mL
1080200R	R1: 4 x 38,6 mL + R2: 4 x 11,4 mL
1080050MK	R1: 1 x 40 mL + R2: 1 x 10 mL

INTENDED USE

Diagnostic reagent for quantitative in *vitro* determination of Direct Bilirubin in serum or plasma on photometric systems.

SUMMARY [1,2]

Bilirubin is a breakdown product of hemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucoronic acid and the resulting water soluble bilirubin glucoronic acid is excreted via the bile ducts. Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60 – 70% of neonates due to an increased postpartum breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation. Common bilirubin methods detect either total bilirubin or direct bilirubin. Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Therefore, the value of unconjugated bilirubin may be estimated from the difference between total bilirubin and direct bilirubin.

METHOD

Photometric test using 2,4-dichloroaniline (DCA)

PRINCIPLE

Direct bilirubin in presence of diazotized 2,4-dichloroaniline forms a red colored azocompound in acidic solution. [4]

REAGENTS

Components and Concentrations

R1	EDTA-(Titriplex III)	0.1 mmol/L
	NaCl	9 g/L
	Sulfamic acid	<200 mmol/L
R2	2,4-Dichloroaniline	<1 mmol/L
	HCl	<100 g/L
	EDTA-(Titriplex III)	0.1 mmol/L

STORAGE AND STABILITY

Reagent is stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C, protected from light and if contamination is avoided. Do not freeze the reagent!

WARNINGS AND PRECAUTIONS

1. Reagents: Warning. May be corrosive to metals. Keep only in original packaging. Absorb spillage to prevent material damage.
2. Avoid contact with skin and eyes using necessary PPE.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. Eltrombopag medication (active ingredient of the drug Revolade®) leads to falsely low or high results in patient samples.
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

WASTE MANAGEMENT

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

The reagent is ready to use.

MATERIALS REQUIRED, BUT NOT PROVIDED

1. NaCl solution 9 g/L.
2. General laboratory equipment.

SPECIMEN

Human serum or heparin plasma
It is very important to store the sample protected from light!

Stability [3]:	2 days	at	20 - 25 °C
	7 days	at	4 - 8 °C
	6 months	at	-20 °C
	in case of immediate freezing.		
	Only freeze once!		

Discard contaminated specimens!

ASSAY PROCEDURE

Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

Wavelength	546 nm, (540 – 560 nm)
Optical path	1 cm
Temperature	20 - 25ºC / 37 ºC
Measurement	Against the reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	50 µL
Distilled water	50 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate for 3 - 5 min at 20 - 25 °C/37 °C, read the absorbance A1, then add:		
Reagent 2	250 µL	250 µL
Mix, incubate for exactly 5 min at 37 °C or 10 min at 20 - 25 °C, then read the absorbance A2.		

ΔA = [(A2 - A1) sample or calibrator] - [(A2 - A1) blank]

CALCULATION

With calibrator

$$\text{Bilirubin Direct [mg/dL]} = \frac{\Delta A_{Sample}}{\Delta A_{Cal.}} \times \text{Conc Cal. [mg/dL]}$$

Conversion Factor
Bilirubin Direct [mg/dL] x 17.1 = Bilirubin Direct [µmol/L]

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. Use Kovalent Topkon N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

PERFORMANCE CHARACTERISTICS

Measuring range

The assay is designed to determine bilirubin concentrations within a measurement range of 0.1 – 10 mg/dL. When the values exceed this range, the samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity / Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, natoproxen up to 1 mmol/L and lipemia up to 1000 mg/dL of triglycerides. Hemoglobin interference occurs from 50 mg/dL. For more information on interfering substances, see Young DS [5].

Sensitivity / Limit of Detection

The lowest detection limit is 0,1 mg/dL.

Precision

Intra-assay precision n = 10	Mean [mg/dL]	SD [mg/dL]	CV [%]
Normal control	0.85	0.03	3.33
Pathological control	1.421	0.04	2.47

Inter-assay precision n = 9	Mean [mg/dL]	SD [mg/dL]	CV [%]
Normal control	0.85	0.01	1.18
Pathological control	1.43	0.02	1.61

Method comparison

Method comparison between Kovalent Bilirrubina Direta (y) and a commercial test (x) using 30 samples demonstrated the following results:
$y = 0.9999 x + 0.0027$; $R^2 = 0.9957$

REFERENCE RANGE [1]

Adults and children ≤ 0.2 mg/dL (≤ 3.4 µmol/L)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LITERATURE

1. Thomas L ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998:192-202.
2. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors, Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.
4. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962; 6:570-8.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mucke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45(9):1240-1243.

CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	In vitro diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:

Kovalent do Brasil Ltda.
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São Gonçalo – RJ – CEP 24722-414 - Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310048	R1: 2 x 50 mL + R2: 2 x 12,5 mL
80115310048	R1: 2 x 40 mL + R2: 2 x 10 mL
80115310048	R1: 3 x 40 mL + R2: 3 x 10 mL
80115310048	R1: 4 x 40 mL + R2: 4 x 10 mL

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Expiration date and Lot no.: See label