BIOLABO
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DIRECT BILIRUBIN Sulfanilic Acid Method

Reagent for quantitative determination of Direct Bilirubin in human serum and plasma.

I REF K1553	**R1** 5 x 18 mL	**R2** 1 x 6 mL
I REF K2553	**R1** 3 x 40 mL	**R2** 1 x 8 mL

TECHNICAL SUPPORT AND ORDERS
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Latest revision : www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (automated method).
It allows the quantitative determination of Direct Bilirubin in human serum and plasma to screen its level.

GENERALITIES (1) (6)

At least four distinct bilirubin species exist in the serum: Direct-reacting bilirubin (DB) consists of mono and di-conjugated bilirubin (β and γ-Bilirubin) and the δ-fraction which is bilirubin tightly bound to albumin; unconjugated α-bilirubin which is water soluble and bound to albumin. Total bilirubin (TB) is the sum of these different species.

PRINCIPLE (4) (5)

Reaction between bilirubin and diazotized sulfanilic acid which leads to a compound, the azobilirubin, colored in very acid or basic medium.

Malloy-Evelyn principle modified by Walters and al: in an aqueous solution, only DB reacts.

The absorbance of azobilirubin thus produced is proportional to the concentration of bilirubin and is measured at 550 nm (530-580).

REAGENTS

R1 BD1 Direct Bilirubin

Sulfanilic acid	30	mmol/L
Hydrochloric acid	130	mmol/L

EUH210: Safety Data Sheet available on request
EUH208: Contains sulfanilic acid. May produce an allergic reaction

R2 BD1 Nitrite Solution

Sodium Nitrite	0.74	mmol/L

According to 1272/2008/EC regulation, these reagents are not classified as dangerous.

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Ready for use.

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert:

Unopened:
- Until expiry date stated on the label of the kit.

Once opened:
- Reagents are stable for at least 1 year at 2-8°C when free from contamination.

Discard any reagent if cloudy or if absorbance at 546 nm > 0.100.

SPECIMEN COLLECTION AND HANDLING (2) (7)

Unhemolysed serum or plasma.
Bilirubin is photo labile. Store the specimen away from light.
Stability in the specimen:

- 4 to 7 days at 2-8°C, 2 days at Room Temperature.

Pediatric or icteric specimen: Refer to specific application.

LIMITS (3)

The bilirubin diazo reaction is temperature sensitive and should be carried out at a constant temperature.
For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Biochemistry Clinical Analyzer KENZA ONE, KENZA 240TX/ISE or KENZA 450TX/ISE

REFERENCE INTERVALS (2)

Adult (and child > 5 days)	Direct Bilirubin	
	mg/dL	[µmol/L]
> 5 days-60 years	< 0.2	[< 3.4]
60-90 years	< 0.2	[< 3.4]
> 90 years	< 0.2	[< 3.4]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

On KENZA ONE, 546 nm, 37°C

Detection limit: approx. 0.04 mg/dL

Linearity Range: between 0.60 mg/dL (QL) and 8.0 mg/dL

Precision:

Within-run N = 20	*Level 1*	*Level 2*	*Level 3*
Mean (mg/dL)	0.80	1.18	1.83
S.D. mg/dL	0.02	0.02	0.02
C.V. %	2.4	1.7	0.9

Between run N = 20	*Level 1*	*Level 2*	*Level 3*
Mean (mg/dL)	0.91	1.29	1.87
S.D. mg/dL	0.04	0.04	0.07
C.V. %	4.5	3.4	3.5

Analytical sensitivity: approx. 0.089 abs for 1 mg/dL

Comparison studies with commercially available reagent:
Study realized on specimens (n=92) between 0.6 and 7.4 mg/dL

y = 0.9981 x – 0.0014 r = 0.9975

Interferences:

Turbidity	Negative interference from 0.090 OD
Ascorbic acid	Positive interference from 713 mg/dL
Hemoglobin	Negative interference from 81 µmol/L
Glucose	No interference up to 1046 mg/dL

Other substances may interfere (see § Limits)

On-board stability: 2 separate reagents are stable 45 days

Calibration Frequency: 45days

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

Results with pediatric method are available on request.

Performances and stability data on KENZA 240TX/ISE and KENZA 450TX/ISE are available on request.

CALIBRATION (8)

- REF 95015 Multicalibrator *traceable to internal master lot (issued from SRM 916).*

The calibration frequency depends on proper instrument functions and on preservation of reagents

QUALITY CONTROL

- REF 95010 EXATROL-N Level 1
- REF 95011 EXATROL-P Level 2
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

PROCEDURE

Refer to validated application of the KENZA Analyzer used

CALCULATION

The analyzer provides directly final result.
Refer to the instruction of use of KENZA analyzer.

REFERENCES

(1) *TIETZ N.W. Textbook of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1133-1137.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 172-177.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-90 to 3-110*
(4) *MALLOY H.T., EVELYN K., J Biol. Chem.(1937), 119, p.481-490*
(5) *WALTERS M, GERARDE H, Microchem J (1970) 15 p.231-243*
(6) *BERNARD S., Biochimie clinique, 2ème éd. Maloine, (1989), p.127-129 et p.280-282*
(7) *Henry RJ, Clin Chem: Principles and technics. Harper and Row. p.592(1965).*
(8) *SRM: Standard Reference Material ®*

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute wih