BIOLABO
www.biolabo.fr

MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

TOTAL AND DIRECT BILIRUBIN

Sulfanilic Acid Method

Reagent for quantitative determination of Total Bilirubin (DMSO as accelerator) or Direct Bilirubin in human serum and plasma.

REF 80403 **R1** 1 x 200 mL **R2** 1 x 200 mL **R3** 1 x 40 mL	REF 80443 **R1** 2 x 200 mL **R3** 1 x 40 mL	REF 80553 **R2** 2 x 200 mL **R3** 1 x 40 mL

TECHNICAL SUPPORT AND ORDERS

Tel: (33) 03 23 25 15 50

Fax : (33) 03 23 256 256
support@biolabo.fr
Latest revision : www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

INTENDED USE

I This reagent is designated for professional use in laboratory (automated method).
It allows the quantification of Total and Direct Bilirubin in human serum and plasma.

GENERALITIES (1) (6)

At least four distinct bilirubin species exist in the serum: Direct-reacting bilirubin (DB) consists of mono and diconjugated 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 diazotised sulfanilic acid which leads to a compound, the azobilirubin, coloured in very acid or basic medium.

Malloy-Evelyn principle modified by Walters and al: in an aqueous solution, only DB reacts. To enable the assay of TB, it is necessary to break the link between unconjugated bilirubin and albumin. This step is done by adding dimethyl sulfoxide (DMSO).

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

REAGENTS COMPOSITION

R1 TOTAL BILIRUBIN

Sulfanilic acid	30	mmol/L
DMSO	7	mol/L
Hydrochloric acid	130	mmol/L

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

R2 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

R3 TOTAL AND DIRECT BILIRUBIN

Sodium Nitrite	0.74	mmol/L

According to 1272/2008 regulation, this reagent is 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.

I 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 and once opened (when free from contamination):

- Until expiry date stated on the label of the kit
- Discard any reagent if cloudy or if absorbance at 550 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 RT.

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. Spectrophotometer or Biochemistry Clinical Analyzer

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 with

CALIBRATION (8)

- Factor as indicated § **CALCULATION** or
- 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

EXPECTED VALUES (2)

Total Bilirubin	mg/dL		[µmol/L]	
New-born	Premature	Full-term	Premature	Full-term
In cord	< 2.0	< 2.0	[< 34]	[< 34]
0-1 day	< 8.0	1.4-8.7	[< 137]	[24-149]
1-2 days	< 12.0	3.4-11.5	[< 205]	[58-197]
3-5 days	< 16.0	1.5-12.0	[< 274]	[26-205]

Adult	Total Bilirubin		Direct Bilirubin	
(child> 5 years)	mg/dL	[µmol/L]	mg/dL	[µmol/L]
> 5 days-60 years	0.3-1.2	[5-21]	< 0.2	[< 3.4]
60-90 years	0.2-1.1	[3-19]	< 0.2	[< 3.4]
> 90 years	0.2-0.9	[3-15]	< 0.2	[< 3.4]

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

PERFORMANCES

On Kenza 240TX, 546 nm, 37°C

Detection limit:
TB: approx. 0.28 mg/dL, DB: approx. 0.01 mg/dL
Linearity Range:
TB: between 0.45 and 20 mg/dL, DB: between 0.60 and 8.0 mg/dL
Precision TB:

Within-run N = 20	*Level 1*	*Level 2*	*Level 3*
Mean (mg/dL)	1.06	3.14	14.14
S.D. mg/dL	0.03	0.06	0.12
C.V. %	2.5	2.0	0.9

Between run N = 20	*Level 1*	*Level 2*	*Level 3*
Mean (mg/dL)	1.03	3.15	13.73
S.D. mg/dL	0.05	0.10	0.37
C.V. %	4.9	3.3	2.7

Precision DB:

Within-run N = 20	*Level 1*	*Level 2*	*Level 3*
Mean (mg/dL)	0.76	1.10	2.06
S.D. mg/dL	0.02	0.02	0.03
C.V. %	2.8	2.2	1.3

Between run N = 20	*Level 1*	*Level 2*	*Level 3*
Mean (mg/dL)	0.74	1.08	2.01
S.D. mg/dL	0.02	0.04	0.05
C.V. %	2.6	3.5	2.5

Analytical sensitivity:

TB: approx. 0.0853 abs for 1 mg/dL

DB: approx. 0.0819 abs for 1 mg/dL Comparison studies with commercially available reagent:
TB: Clinical evaluation (n=96) between 0.31 and 9.26mg/dL
y = 0,9446 x – 0,0546 r = 0.9960

DB: Realised on specimens (n=92) between 0.1 and 7.4 mg/dL
y = 0.9981 x – 0.0001 r = 0.9975

Interferences:	Total Bilirubin	Direct Bilirubin
Turbidity	Negative interference from 0.052 OD	Negative interference from 0.067 OD
Ascorbic acid	No interference up to 2500 mg/dL	No interference up to 2500 mg/dL
Haemoglobin	No interference up to 385 µmol/L	Negative interference from 57 µmol/L
Glucose	No interference up to 1059 mg/dL	No interference up to 1059 mg/dL

Other substances may interfere (see § Limits)

On-board stability: 2 separate reagents are stable 60 days

Calibration Frequency: 60 days,
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 paediatric method are available on request.

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. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. **With factor:** Verify analysis parameters (Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor).
4. Use a new vial of reagent and repeat the test.
5. **With a calibrator:** If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
6. If control is still out of range, calibrate again with a new vial of reagent and repeat the test.

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

MANUAL PROCEDURE

Let stand reagents and specimen at room temperature

	Assay	Blank
Reagent R1 (TB) or R2 (DB)	1000 µL	1000 µL
Distilled water		50 µL
Reagent R3	50 µL	
Mix and add		
Specimen, calibrator or control	100 µL	100 µL

Zero on distilled water and drain well the cuvette. Read first all blanks of one run then all the assays, well-draining cuvette between each tube. But DO NOT RINSE WITH WATER as it could produce streaks on the cuvette.

Mix well and start a timer when adding specimen. Read absorbance at 550 nm (530-580) against blanks

TB: reading after > 3 minutes at 37°C or > 5 minutes at room temperature

DB: reading at exactly 3 minutes at 37°C or 5 minutes at room temperature

1- Performances with manual procedure should be validated by user.
2- Kenza applications and other applications proposal are available on request.
3- Paediatric Specimen: divide sample volume by 5

CALCULATION

With Seric Muticalibrator

$$\text{Result} = \frac{\text{Abs (Assay - Blank) Specimen}}{\text{Abs (Assay - Blank) Calibrator}} \times \text{calibrator concentration}$$

With Factor (Path length 1 cm, 37°C, 550 nm)

mg/dL = [Abs. assay – Abs. Blank] x 11.4*
µmol/L = [Abs. assay – Abs. Blank] x 195*

With Paediatric method: multiply the factor by 5 (sample volume).

*This factors should be used as a guide only

REFERENCES

(1) *TIETZ N.W. Text book 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 ®*