BILIRUBIN LR liquid reagent

REF 1752125
Direct Bilirubin R1 1x100 ml R2 1x25 ml
Total Bilirubin R1 1x100 ml R2 1x25 ml

CE IVD For in vitro medical device

Use
Kit for measurement of direct bilirubin in serum or plasma. Colorimetric method Jendrassik - Grof modified.

Summary
Direct bilirubin measurements are used in the diagnosis and treatments of various liver diseases, and metabolic disorders.

Principle
End point analysis. Direct (conjugated) bilirubin reacts with sulphanilic acid and giving a coloured azocompound (azobilirubin). The increase in absorbance due to the formation of azobilirubin is proportional to the direct bilirubin concentration in the sample.

Reagents
R1 Sulphanilic acid 22.0 mmol/l preservatives and surface-active agents not anionic
R2 Nitrite sodium 0.35 mmol/l

Reagent Preparation
Reagents are liquid and ready to use. About using as monoreagent ("sample-starter") add to every 4 ml of **R1** reagent, 1 ml of **R2** reagent.

Storage And Stability
- Store the kit at 15-25°C. Do not freeze the reagents.
- After opening, the vials R1 and R2 are stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.
- Working solution stability (R1+ R2): 14 days at 2-8°C.

Precaution In Use
The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). The final concentration of the components is below the limits imposed by Regulation (EC) No. 1272/2008 - CLP (and subsequent amendments) and Directive 88/379/CEE and subsequent amendments to the classification-packaging and labeling of dangerous substances.
However the reagent should be handled with care, according to good laboratory practice.

Waste Management
Please refer to the local legal requirements.

Sample
- Serum or EDTA-Na_2 plasma.
- Do not use samples with haemolysis.
- Keep the samples far from light and heat because the bilirubin is a photosensitive pigment.
- Perform the samples as soon as possible.

Note
- The kit, according to this method, must be used in manual procedures. About automatic use follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the single vials.

Procedure

Wavelength	λ: 570 (550 – 580) nm
Working Temperature	37°C
Optical Path	1 cm
Reaction	"end point"

Monoreagent Procedure "sample starter"

	Blank	Sample
Working Reagent	1500µl	1500µl
Distilled Water	100 µl	–
Sample	–	100 µl

Mix, then incubate 5' at 37°C. Measure the absorbance of sample (EC) and blank (EBC) against water.

Bireagent Procedure "substrate starter"

	Blank	Sample
Reagent R1	1200µl	1200µl
Distilled Water	100 µl	–
Sample	–	100 µl

Mix, incubate at 37°C for 5' and then add:

Reagent R2	300 µl	300 µl

Mix, then incubate 5' at 37°C. Measure the absorbance of sample (EC) and blank (EBC) against water.

Calculation
Direct bilirubin [mg/dl] = (EC-ECB) x 14.5
Direct bilirubin [µmol/l] = (EC-ECB) x 248

The reagents performances are related to 37°C, 1 cm and 570 nm.

Conversion Factor
Bilirubin [mg/dl] x 17.0 = Bilirubin [µmol/l]

Reference Values
Adults 0 - 0.35 mg/dl (0 – 5.1 µmol/l)

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

Analytical Performances
The performance of the reagent are related to 37 ° C, 1 cm and 570 nm

Linearity
The reaction is linear in concentration range between 0.04 e 10 mg/dl. Samples with values exceeding 10 mg/dl must be diluted with saline solution. Then, multiply, the result for diluting factor.

"Intra-Assay" precision (within-Run)
Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (mg/dl)	N = 0.82	H = 2.32
S.D.	N = 0.02	H = 0.06
C.V.%	N = 2.67	H = 2.62

"Inter-Assay" precision (between-Run)
Determined on 20 samples for each control (N-H).Results:

MEAN (mg/dl)	N = 0.82	H = 2.31
S.D.	N = 0.02	H = 0.05
C.V.%	N = 2.69	H = 1.96

Analytical sensitivity
The test sensitivity in terms of detection limit is 0.04 mg/dl.

Correlation
A study based comparing this method with a similar method on 2 samples has given a correlating factor **r = 0.99**

y = 1.0036x + 0.0333

Interferences
No interference was observed by the presence of

Triglycerides	≤ 500 mg/dl
Haemoglobin	≤ 200 mg/dl

For a comprehensive review of interfering substances, refer to the publication by Young.

Quality Controls
It's necessary, every time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography

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Young D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Washington, DC 5th ed.2000.

Simbols
CE CE Mark (requirement of 98/79 regulation)
IVD in vitro medical device
LOT Batch Code
Use by
Storage temperature limits
Read instruction for use
Gesan Production srl

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