DIRECT BILIRUBIN

Colorimetric method with sample blank

Liquid Reagents ready to use

REF. 0032/50 4x 50 ml
REF. 0032/2 2x100 ml
REF. 0032 4x100 ml

CE IVD

Azienda certificata DNV

INTENDED USE

Quantitative determination of Direct Bilirubin in serum.

PRINCIPLE

In an acid medium, direct bilirubin reacts with diazotized sulphanilic acid to form a pink diazo compound (azobilirubin), whose intensity is proportional to the concentration of direct bilirubin present in the sample.

SAMPLE

Not hemolyzed fresh serum.
Analyze samples within 2 hours after collection. Protect samples from light.
Direct sunlight may cause up to 50% decrease in direct bilirubin within 1hour.
The samples are stable 3 days at 2-8°C in the dark and one month at -20°C.

KIT COMPONENTS

Reagent (A) BIL-D Volume = 50/100 ml	Sulfanilic Acid hydrochloric acid 23 %	40 mmol/l 270 mmol/l
Reagent (B) BIL-D Volume = 25 ml	Sodium nitrite	143 mmol/l

The reagents are stable until the expiration date when stored at temperatures as indicated on the label and protected from light.
Once opened reagents are stable for 2 months if contamination is avoided.
Keep bottles closed when not in use.

REAGENT PREPARATION

Liquid Reagents ready to use.
For **use as Monoreagent**: combine the Reagent (B) and (A) in the ratio 1:61. Add 0.5 ml of Reagent (B) to 30 ml of Reagent (A).
The working solution (A+B) is stable at least 5 days at room temperature (15-25°C) in a dark bottle, protected from light.

PRECAUTIONS AND WARNINGS

The Reagent (A) contains sulfanilic acid: may produce an allergic reaction.
The Reagents may contain some non-reactive and preservative components.
It is suggested to handle carefully it, avoiding contact with skin and swallow.
Use the normal precautions required in the laboratory.
Dispose of waste according to local laws.

PROCEDURE

Wavelength:	546 nm (550)
Lightpath :	1 cm
Temperature:	25, 30, 37°C
Reading:	against reagent blank and sample blank
Method:	Increasing End Point

Use as monoreagent:

pipette:	reagent blank	sample	sample blank
Reagent (A+B)		1500 µl	
Reagent (A)	1500 µl		1500 µl
sample		100 µl	100 µl
water	100 µl		

Use as bi-reagent:

Prepare two series of test tubes, one for the measurement of the samples blank, the other for the measurement of the samples absorbance.

pipette:	reagent blank	sample	sample blank
Reagent (A)	1400 µl	1400 µl	1400 µl
Reagent (B)	20 µl	20 µl	
sample		120 µl	120 µl
water	120 µl		20 µl

Mix, incubate at 25, 30, 37°C for 5 minutes and read against reagent blank the sample absorbance (Ax) and the blank sample absorbance (Abx).

Reaction volumes can be proportionally modified.
This method describes the manual procedure to use the kit.
For automated procedure, ask for specific applications.

RESULTS CALCULATION WITH FACTOR (BI-REAGENT)

Direct Bilirubin mg/dl = (Ax – Abx) x 14.9

RESULTS CALCULATION WITH CALIBRATOR

Direct Bilirubin mg/dl = (Ax – Abx)/(Ac – Abc) x Calibrator Value

Factor to convert mg/dl in µmol/l = 17.1

EXPECTED VALUES

adults: ≤ 0.25 mg/dl (≤ 4.3 µmol/l)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL

You must perform the controls at each kit's use and verify that the values obtained are within the reference range reported in the operating instructions. For this purpose we recommend the use of control sera: PRECISENORM (REF.6000) and PRECISEPATH (REF.6001).

PERFORMANCE

Sensitivity: the sensitivity of the method is: 0.05 mg/dl.
Linearity: the method is linear up to 20 mg/dl. For higher values, dilute the sample 1:2 and multiply the result by 2.
Precision intra-assay:

	Level 1	Level 2	Level 3
Mean (mg/dl)	0.22	0.58	5.38
DS	0.008	0.011	0.062
CV %	3.58	1.86	1.15

Precision inter-assay:

	Level 1	Level 2	Level 3
Mean (mg/dl)	0.21	0.58	5.47
DS	0.007	0.007	0.060
CV %	3.51	1.17	1.09

Interferences: The triglycerides do not interfere up to 1000 mg/dl.
Hemoglobin does not interfere up to 150 mg/dl.
Correlation against a reference method: Y = 1.0453x + 0.0062 r = 0.9844

REFERENCES

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