

DIRECT BILIRUBIN

Colorimetric method with sample blank
Liquid Reagents ready to use

REF 12101160 R1 8X20mL
REF 11201020 R2 4X5mL

IVD

PRODUCT CODE: BIL-8D25

Company with
Quality System
ISO 9001 – ISO 13485
Certified by LMS



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)	280 µl	280 µl	280 µl
Sample		20 µl	20 µl
Water	20 µl		
Reagent (B)	25 µl	25 µl	

Mix, incubate at 37°C for 2 minutes and read against reagent blank the sample absorbance (Ax) and the blank sample absorbance (Abx).

RESULTS CALCULATION WITH FACTOR (BI-REAGENT)

Direct Bilirubin mg/dl = $(Ax - Abx) \times 17.7^*$

*Variable factor depending on the application method and/or instrument

RESULTS CALCULATION WITH CALIBRATOR

Direct Bilirubin mg/dl = $(Ax - Abx) / (Ac - Abc) \times \text{Calibrator Value}$
Factor to convert mg/dl in µmol/l = 17.1

EXPECTED VALUES

adults: $\leq 0.25 \text{ mg/dl}$ ($\leq 4.3 \text{ µmol/l}$)

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 Sicurobio control sera: ACUTROL 1 (REF. SB-8334) and ACUTROL 2 (REF. SB-8335).

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

1. Vassault, A. et al. Ann. Biol. Clin., 44,686 (1986).
2. J.A. Lott and B.T. Doumas, Clin. Chem. 641-647, 39 (1993).
3. Young D. S., et al, Clin. Chem. 21:1D (1975).
4. BurtisCA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed., Liver Function.

INTENDED USE

Quantitative determination of Direct Bilirubin in serum.

CLINICAL SIGNIFICANCE

Bilirubin is a yellow pigment derived from the catabolism of the heme group (mainly from senescent red blood cells). It exists in two forms: indirect (unconjugated) bilirubin, which is transported to the liver bound to albumin, and direct (conjugated) bilirubin, which in the liver is conjugated with glucuronic acid, is water-soluble, and excreted with bile. An increase in direct bilirubin values may indicate liver disease, cholestasis, or biliary obstruction.

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 1 hour.

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 = 100 ml	Sulfanilic Acid Hydrochloric acid	40 mmol/l 270 mmol/l
Reagent (B) BIL-D Volume = 50 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. Do not use over expiry date.

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.

PRECAUTIONS AND WARNINGS

Reagent (A):

EUH210 Safety data sheet available on request.

EUH208 Contains: SULFONIC ACID

May produce an allergic reaction.

Reagents may contain some non-reactive and preservative components. Use the normal precautions required in the laboratory.

Dispose of waste according to local laws.

PROCEDURE

Wavelength: 546 nm
Lightpath: 1 cm
Temperature: 25, 37°C
Reading: against reagent blank and sample blank

Method: End Point with Sample Blank