



GENESIS

Instructions For Use

Bilirubin (DMSO T&D)

Cat no.	size
1102 201	2*100
1102 202	2*60

INTENDED USE

Bilirubin Reagent is intended for in vitro Quantitative diagnostic determination of total and direct bilirubin.

DIAGNOSTIC CHARACTERISTICS

Bilirubin is a waste product derived from the heme moiety of the hemoglobin released from senescent or damaged erythrocytes that are destroyed in the Reticuloendothelial cells. After production, bilirubin is transported to the liver in association with albumin. Inside the hepatocytes bilirubin is conjugated with glucuronic acid and it is excreted into bile. A number of inherited and acquired diseases affect production, uptake, metabolism, and excretion of bilirubin, resulting in hyperbilirubinemia. Unconjugated hyperbilirubinemia is seen in newborns (physiological jaundice), in increased red cell destruction (hemolytic anemia, extensive hematoma), in ineffective erythropoiesis and in some rare genetic diseases (Gilbert's syndrome, Crigler-Najjar syndrome). Conjugated hyperbilirubinemia is associated to a decreased excretion of bile due to liver diseases (hepatitis or cirrhosis) or to intrahepatic or extrahepatic cholestasis.

Jaundice is a clinical manifestation of hyperbilirubinemia, consisting of deposition of bile pigments in the skin, resulting in a yellowish staining of the skin and mucous membranes.

PRINCIPLE OF THE METHOD

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin glucuronide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin the direct is also determined; the results correspond to total bilirubin. The intensity of the color formed is proportional to the bilirubin concentration in the sample.

TOTAL BILIRUBIN

Bilirubin + Diazotized Sulfanilic acid + NaNO₂ $\xrightarrow{\text{DMSO}}$ Azobilirubin

DIRECT BILIRUBIN

Bilirubin + Diazotized Sulfanilic acid + NaNO₂ \longrightarrow Azobilirubin

COMPOSITION

TOTAL BILIRUBIN (R ₁)	Sulfanilic acid	5 mmol/L
	HCL	0.3 N
	DMSO	5 mol/L
DIRECT BILIRUBIN (R ₂)	Sulfanilic acid	5 mmol/L
	HCL	0.3 N
SOD NITRITE (R ₃)	Sodium nitrite	7 mmol/l

STORAGE.

Store at 2-8°C.

Reagents are stable until the expiry date shown on the Vial label.

REAGENT PREPARATION

Reagents provided are ready to use.

ADDITIONAL EQUIPMENT

Analyzer, spectrophotometer or photometer able to read at 546nm

SPECIMEN

1. Serum collected by standard procedures.
2. Bilirubin in serum is stable for 2 days at 2-8°C if serum is protected from light.
3. Discard contaminated specimens.

Symbols in Product Labeling			
EC REP	Authorized Representative		Expiration date
IVD	For in-vitro diagnostic use		CAUTION, consult instructions for use
REF	Catalogue number		Manufactured by
LOT	Lot number		

Precautions and Warnings

After contact with skin, wash immediately with plenty of soap and water. Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

PROCEDURE

	Total Bilirubin		Direct Bilirubin	
	Blank	Sample	Blank	Sample
TOTAL BILIRUBIN (R ₁)	1.0 ml	1.0 ml	-	-
DIRECT BILIRUBIN (R ₂)	-	-	1.0 ml	1.0 ml
Sodium Nitrite (R ₃)	-	50 µL	-	50 µL
Sample	50 µL	50 µL	50 µL	50 µL

Mix thoroughly and incubate the tubes for exactly **5 minutes** at room temperature (16-25°C).

Measure the absorbance (A) of the Sample at 546 nm against the sample blank.

CALCULATIONS

The Bilirubin concentration in the sample is calculated using the following general formula:

Total bilirubin (mg/dl) = (A sample – A sample blank) x 20.0

Direct bilirubin (mg/dl) = (A Sample – A sample blank) x 14.0

REFERENCE VALUES

Total Bilirubin	Up to 1.0 mg/dL = 17 µmol/L
Direct Bilirubin	Up to 0.2 mg/dL = 3.4 µmol/L

METROLOGICAL CHARACTERISTICS

Detection limit (Total Bilirubin): 0.03 mg/dL.
 (Direct Bilirubin): 0.02 mg/dL
 Linearity limit: 18.0 mg/dL.

- Repeatability (within run):

Total Bilirubin			Direct Bilirubin		
Mean Concentration	cv	n	Mean Concentration	cv	n
0.59 mg/dL	3 %	20	0.77 mg/dL	1.2 %	20
6.74 mg/dL	1%	20	1.36 mg/dL	0.5%	20

- Reproducibility (run to run):

Total Bilirubin			Direct Bilirubin		
Mean Concentration	cv	n	Mean Concentration	cv	n
0.59 mg/dL	3.6 %	20	0.77 mg/dL	2.3 %	20
6.74 mg/dL	3.3%	20	1.36 mg/dL	0.9%	20

INTERFERENCES

Hemoglobin (10 g/L) does not interfere.

Lipemia (triglycerides > 15 g/L) may interfere. Other drugs and substances may interfere

BIBLIOGRAPHY

1. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Brunis DE. WB Saunders Co, 2005.

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