Direct Bilirubin

DMSO. Colorimetric

IVD CE

PRODUCT CODE
CS025

INTENDED USE
The reagent is intended for in vitro quantitative determination of Direct Bilirubin in serum or plasma.

CLINICAL SIGNIFICANCE
Bilirubin is a breakdown product of hemoglobin.
It is transported from the spleen to the liver and excreted into bile.
Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma. Causes of hyperbilirubinemia:
Total bilirubin: Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythrpoiesis, and drugs.
Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage1,6,7
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE
Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin-glucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulphoxide (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 sample1,2,3

REAGENT COMPOSITION

Direct Bilirubin Reagent (R1)	
Sulphanilic Acid	30 mmol/L
Hydrochloric acid (HCl)	150 mmol/L
Direct Bilirubin, Nitrite Reagent (R2)	
Sodium nitrite	29 mmol/L

PREPARATION
Both reagents are ready to use.

REAGENT STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Color development in R 2.

SPECIMEN
-Serum or plasma, free of hemolysis
-Protect samples from direct light. Stability: Bilirubin is stable at 2-8ºC for 4 days and 2 months at –20ºC.

PRECAUTION
R1: H290-May be corrosive to metals. H314-Causes severe burns and eye damage. EUH208-Contains sulphanilic acid. May produce an allergic reaction.

PROCEDURE

1.ASSAY Condition

Wavelength	555 nm (530-580)
Temperature	15-25 °C
Measurement	Against sample blank (without nitrite)

2. Adjust the instrument to zero with distilled water.
3.Pipette into a cuvette

	Blank	Sample
direct Bilirubin Reagent (R1)	1000 µL	1000 µL
direct Bilirubin, Nitrite reagent (R2)	--	50 µL
Sample	100 µL	100 µL

4.Mix and stand for exactly '10' minutes at room temperature.
5. Measure the absorbance of sample Blank (As).

CALCULATION

-With Calibrator:

$$\frac{(A)Sample - (A)Sample\ Blnk}{(A)Calibrator - (A)Calibrator\ Blank}\ x\ Conc.Calibrator = mg/dl\ of\ bilirubin\ in\ the\ sample$$

-With Factor :

$$((A)\boldsymbol{Sample} - (A)\boldsymbol{Sample\ \ Blank})\boldsymbol{x\ Factor} = \boldsymbol{mg/dl}\ \ of\ bilirubin\ in\ the\ sample$$

$$Factor = \frac{\text{Concentration of Calibration}}{(A)Calibrator - (A)Calibrator\ \ Blank}$$

Conversion Fctor : $\boldsymbol{mg/dl\ x\ 17.1\ \mu\ mol}$.

NORMAL RANGE
Bilirubin Direct: Up to 0,25 mg/dL ≅ 4,27 µmol/L
These values are for orientation purpose; each laboratory should establish its own reference range

QUALITY CONTROL
All control sera with Direct Bilirubin value estimated by this method can be used.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

LINEARITY
From detection limit of 0,07 mg/dL to linearity limit of 20 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

BIBLIOGRAPHY

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