Total Bilirubin

IVD CE

DMSO. Colorimetric

PRODUCT CODE
CS024

INTENDED USE
The reagent is intended for in vitro quantitative determination of Total 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 sample

REAGENT COMPOSITION

Total Bilirubin Reagent (R1)	
Sulphanilic Acid	30 mmol/L
Hydrochloric Acid	50 mmol/L
Dimethylsulphoxide (DMSO)	7 mol/L
Total Bilirubin, Nitrite Reagent (R2)	
Sodium Nitrite	29 mmol/L

REAGENT 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
Fresh hemolysis-free serum or heparinized plasma may be used. Carefully protect from light until use. Bilirubin in sample is stable for '4' days when stored in the dark at 2-8° C 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. Follow the precautionary statements given in MSDS and label of the product.
- To avoid contamination, use clean laboratory wares.
- Avoid direct exposure of reagent to light.

PROCEDURE

1. Assay condition:

Reaction type	End point
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
Total Bilirubin Reagent (R1)	1.5ml	1.5ml
Total 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:**

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

-With Factor:

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

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

$$Conversion\ factor: mg/dl\ x\ 17.1\ \mu mol/L$$

NORMAL RANGE
Bilirubin Total in adult Up to 1,10 mg/dL ≅ 18,81 µmol/L
Total Bilirubin in newborn <12 mg/dl ≅ 205.2 µmol/L
It is recommended that each laboratory establishes its own reference range

LINEARITY
From detection limit of 0,00526 mg/dL to linearity limit of 18 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.

QUALITY CONTROL
All control sera with Total 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.

NOTES
1- It is important to ensure the working reagent and nitrite reagent are thoroughly mixed before adding the sample.
2- Bilirubin levels may be reduced if the sample is exposed to light. Haemolytic sample will also show low value.

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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EC REP

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