

**PRODUCT CODE**  
**CS003**

**INTENDED USE**

The reagent is intended for in vitro quantitative determination of Total Bilirubin in serum or plasma.

**CLINICAL SIGNIFICANCE**

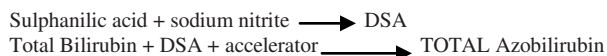
Bilirubin is formed by the breakdown of RBC's in the spleen, liver & bone marrow. Small amount of bilirubin circulates in the plasma loosely bound to albumin, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated with glucuronic acid, which forms a soluble compound. This is referred to a direct bilirubin.

Elevated levels are found in Hepatitis, Cirrhosis, Haemolytic jaundice, obstruction of biliary tract & drug induced reactions.

**PRINCIPLE**

Bilirubin reacts with Diazotized Sulphanilic Acid (DSA) to form a red azo dye, the intensity of which at 546 nm is directly proportional to the bilirubin concentration in the sample. Water-soluble bilirubin glucuronides react "directly" with DSA whereas the free or "indirect" bilirubin will only react with DSA in presence of an accelerator.

Total - Direct = Indirect bilirubin



**REAGENT COMPOSITION**

**Total Bilirubin Reagent (R1)**

Sulphanilic Acid	14 mmol/L
Hydrochloric Acid	250 mmol/L
Caffeine (accelerator)	200 mmol/L
Sodium Benzoate	420 mmol/L

**Total Bilirubin, Nitrite Reagent (R2)**

Sodium Nitrite	6.5 mol/L
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**REAGENT PREPARATION**

Both reagents are ready to use.

**REAGENT STORAGE AND STABILITY**

Reagent 1 and Reagent 2 are stable to the given expiry date if stored at 15-25°C.

**SPECIMEN**

Fresh hemolysis-free serum or heparinized plasma may be used. Carefully protect from light until use. Bilirubin in sample is stable for '3' days when stored in the dark at 2-8° C.

**PRECAUTION**

To avoid contamination, use clean laboratory wares. Avoid direct exposure of reagent to light.

**ASSAY**

Reaction type	:	End point
Wavelength	:	546nm
Temperature	:	20-25°C
Measurement	:	Against sample blank (without nitrite)

**PROCEDURE**

	Blank	Sample
Total Bilirubin Reagent (R1)	1000 µL	1000 µL
Total Bilirubin, Nitrite reagent (R2)	--	20 µL
Sample	100 µL	100 µL
Mix and stand for exactly '5' minutes at room temperature. Measure the absorbance of sample Blank (As).		

**CALCULATION**

Bilirubin Concentration = As X 13.2 mg/dL  
 To convert mg/dL to µmol/L. multiply 17.1

**NORMAL RANGE**

Total Bilirubin

At birth up to	5 mg/dL	85.5 µmol/L
5 days up to	12 mg/dL	205.0 µmol/L
1 month up to	1.5 mg/dL	25.6 µmol/L
Adults up to	1.1 mg/dL	18.8 µmol/L

It is recommended that each laboratory establishes its own reference range

**LINEARITY**

up to 10 mg/dL, the values exceeding 10 mg/dL dilute serum 1+4 with physiological saline (NaCl: 9g/L) and repeat the assay. Multiply the result by 5.















**QUALITY CONTROL**

All control sera with Total Bilirubin value estimated by this method can be used.

**NOTES**

- 1- It is important to ensure the working reagent and nitrite reagent are thoroughly mixed before adding the sample.
- 2- For bilirubin values exceeding 10 mg/dL dilute serum 1+4 with physiological saline (NaCl: 9g/L) and repeat the assay. Multiply the result by 5.
- 3- 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
	Catalogue Number		Pack Size
	Expiry Date		Volume
	Storage Condition		Lot Number
	Instruction for Use		In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests		For Single Use Only
	EC Representative		European conformity

**BIBLIOGRAPHY**

1. Jendrassik, L. and Grof , P : Biochem Z, 297,81 1938.
2. Van der Bergh, A.A. and Muller, P., Biochem Z, 77, 90, 1916.
3. Tietz, N.W., Fundamentals of Clinical Chemistry, p.940. W.B. Saunders Co., Philadelphia , 1987.

