

Reagent kit for quantitative estimation of Total and Direct Bilirubin in Serum or Plasma.

DIAGNOSTICS SIGNIFICANCE:

Total & Direct Bilirubin determination in serum is used for the diagnosis. The conjugated Bilirubin is predominantly increased in obstructive jaundice due to regurgitation while hepatic jaundice is associated with increase in both conjugated & unconjugated Bilirubin in sample.

PRINCIPLE:

Bilirubin reacts with diazotized sulphanilic acid to form an azocompound the colour of which is measured at 546 nm (530-560 nm) and is proportional to the concentration of Bilirubin. For Total Bilirubin the reaction is accelerated by caffeine reagent. The readings for Total & Direct Bilirubin are taken after 2 minutes incubation.

SAMPLE COLLECTION & STORAGE:

Serum is preferred with no hemolysis, Plasma with heparin as anticoagulant may be used.

KIT PRESENTATION:

Pack	R1-Bilirubin (Sulphanilic Acid) Diazo A	R2-Bilirubin (Sodium Nitrite) Diazo B	R3-Bilirubin (Caffeine)
2 X 50 ml	1 X 12 ml	1 X 10 ml	2 X 50 ml
2 X 100 ml	1 X 24 ml	1 X 20ml	2 X 100 ml
4 X 100 ml	1 X 50 ml	1 X 40 ml	4 X 100 ml
10 X 100 ml	1 X 120 ml	1 X 100 ml	10 X 100 ml

STORAGE & STABILITY OF THE REAGENTS:

All the reagents in the kit are stable at RT until expiry date stated on the labels.

ASSAY PARAMETERS:

For Monochromatic		For Bichromatic	
Reaction	: End Point	Reaction	: End Point
Wavelength - I	: 546 nm	Wavelength - I	: 546 nm
Wavelength - II	: NA	Wavelength - II	: 630 nm
Sample Blk	: Yes	Sample Blk	: No
Zero Setting	: Dist. Water	Zero Setting	: Dist. Water
Flow Cell Temp.	: 30 °C	Flow Cell Temp.	: 30 °C
Incubation Time	: 2mins. RT	Incubation Time	: 2mins. RT
Sample Volume	: 50 µl	Sample Volume	: 50 µl
Reagent Volume	: 1.1 ml	Reagent Volume	: 1.1 ml
Factor	: 26.00	Factor	: 26.00
Linearity	: 25 mg/dl	Linearity	: 25 mg/dl
Unit	: mg/dl	Unit	: mg/dl

PROCEDURE:

For Monochromatic Method:

Pipette into TT	Total Bilirubin		Direct Bilirubin	
	Sample Blank	Test	Sample Blank	Test
R1-Bilirubin (Diazo A)	50 µl	50 µl	50 µl	50 µl
R2-Bilirubin (Diazo B)	--	50 µl	--	50 µl
Sample	50 µl	50 µl	50 µl	50 µl
R3-Bilirubin (Caffeine)	1.0 ml	1.0 ml	--	--
Distilled Water	--	--	1.0 ml	1.0 ml

Mix & incubate for 2 minutes at RT & read the absorbance at 546 nm against Sample Blank.

For Bichromatic Method:

Pipette into TT	Total Bilirubin	Direct Bilirubin
Sample	50 µl	50 µl
R1-Bilirubin (Diazo A)	50 µl	50 µl
R2-Bilirubin (Diazo B)	50 µl	50 µl
R3-Bilirubin (Caffeine)	1.0 ml	--
Distilled Water	--	1.0 ml

Mix & Incubate for 2 minutes at RT & read the absorbance at 546 & 630 nm against Distilled Water.

CALCULATION:

For Monochromatic Method

Bilirubin (mg/dl) of Test Sample = (AT – ASB) X Factor

Where AT = Absorbance of Test Sample
ASB = Absorbance of Sample Blank

For Bichromatic Method

Bilirubin (mg/dl) of Test Sample = Abs. of Test X Factor

NORMAL VALUES:

Total Bilirubin : 0.4 – 1.1 mg/dl	Direct Bilirubin : 0.1 – 0.4 mg/dl
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Each laboratory should establish its own reference range.

LINEARITY:

This method is linear up to 25 mg/dl. For sample values higher than 30 mg/dl, dilute the samples suitably with 0.9% saline and repeat the assay. Apply proper dilution factor to calculate the final results.

REFERENCES:

- Jendrassik, L & Grof P. Biochem Z. 297, 81 (1938).
- Schellong, G and Wende, U. Arch Kinderheik 162, 120 (1960).

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 Expiry Date
  In-Vitro Diagnostics Use
  Storage
  Mfg. Date
  Batch Number
  Catalogue Number
  See Package Insert