Pariksha Biotech
A game changer in IVD

Bilirubin (Total) Estimation Kit

High-Q Bilirubin-(Total)

(Jendrassik & Grof Method)

HIGH
Command on Quality

Intented Use:

Kit for the quantitative determination of of Total Bilirubin in serum or Plasma.

Summary:

Total bilirubin concentration reflects the levels of both the conjugated and unconjugated fractions of bilirubin. Total bilirubin levels are elevated in various forms of liver disease such as cirrhosis, hepatitis, and obstructions of the hepatobiliary system such as gallstones or tumors. Elevated total bilirubin levels are also observed in cases of intravascular hemolysis.Detergents in the reagent solubilize free bilirubin from the sample, allowing both the free and conjugated bilirubin to react with diazotized modified 2,4-dichloroaniline to form a red to purple chromogen, which absorbs at 546 nm. This change in absorbance is directly proportional to the total bilirubin concentration of the sample. A sample blank is taken to minimize interference by endogenous chromogens.

Principle:

This reagent is a variation of the classical method of Van den Bergh and Mueller. Total bilirubin, both conjugated and free, is measured by using a stabilized diazonium salt of 2,4-dichloroaniline which reacts with bilirubin to form azobilirubin with maximum absorbance at 540 nm. Surfactants are used as reaction accelerators. The concentration of bilirubin present is directly proportional to the absorbance of the azobilirubin measured spectrophotometrically at 540 nm.

Sample Collection, Storage & Stability:

Serum is the preferred sample. Plasma with heparin as anticoagulant may be used. Serum or Plasma should be separated as early as possible. Samples are stable for a day when stored tightly capped at 2-8ºC and for a month at -10º.Avoid exposure of samples to direct light during processing and storage. Cross contamination at any stage makes the samples unsuitable for use. The samples should be brought to room temperature prior to use. Do not use hemolyzed or cross contaminated samples.

Storage and Stability of the reagents:

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

Presentation of the kit:

All the reagents are ready to use and there is no need to prepare working reagents anywhere

Reagent Composition:

Total Bilirubin Reagent:

Sulphanilic Acid	: 50 mMol/L
Concentrated Hydrochloric Acid	: 150mMol/L
2,4-dichloroaniline	: 0.8 mMol/L

Sodium Nitrite Reagent:

Sodium Nitrite:	: 300 mMol/L

System Parameters for Total Bilirubin (Monochromatic with Sample Blank)

Type of Reaction	:	End Point
Reaction Slope	:	Increasing
Wavelength	:	546 nm
Sample Blank	:	yes
Flowcell Temperature	:	37º C
Incubation time	:	5 min. at R.T.
Factor	:	**25 (Total Bilirubin)**
Sample Volume	:	50µl
Reagent Volume	:	1.050 ml.
Zero setting with	:	Sample Blank

Test Procedure for Total Bilirubin Estimation (Monochromatic Method):

Reagent	Sample Blank	Test (T)
Total Bilirubin Reagent	1.00 ml	1.00 ml
Sodium Nitrite	---	50 µl
Sample	50 µl	50 µl

Mix & incubate for 5 mins. at R.T. & read the absorbance of Test against its sample blank at 546 nm.

Calculation:

Total Bilirubin (mg/dl)= Abs of Test - Abs of Sample Blank x 25

System Parameters for Total Bilirubin - Bichromatic Method (Dual Wavelength)

Type of Reaction	:	End Point
Reaction Slope	:	Increasing
Wavelength	:	546 nm & 630
Flowcell Temperature	:	37º C
Incubation time	:	5 min. at R.T.
Factor	:	**27 (Total Bilirubin)**
Sample Volume	:	50µl
Reagent Volume	:	1.050 ml.
Zero setting with	:	Distilled Water

Test Procedure for Total Bilirubin Estimation: (Bichromatic Method--Dual Wavelength)

Reagent	Test (T)
Total Bilirubin Reagent	1.00 ml
Sodium Nitrite	50 µl
Sample	50 µl

Mix & incubate for 5 mins. at R.T. & read the absorbance of Test against distilled water at 546 & 630 nms

Calculation:

Total Bilirubin (mg/dl)= Abs of Test x 27

Quality Control:

The integrity of the assay should be monitored by the use of control sera (normal and abnormal) with known bilirubin concentrations.

Reference Values:

Total Bilirubin : Adults 0.0 –1.1 mg/dl

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Linearity:
The assay is linear up to 25.0 mg/dl. Samples exceeding linearity should be diluted with normal saline and repeated. Multiply the concentration by the dilution factor

Performance:

1. **Comparison:**
   Testing performed between this and a similar method yielded a coefficient of correlation of 0.987 with a regression equation of y=0.98x + 0.02.

2. **Precision:**

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
0.9	0.05	5.6	0.9	0.05	5.6
7.7	0.05	0.6	7.9	0.14	1.8

Bibliography:

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2. Annino, J.S., Clinical Chemistry Principles and Procedures, 2nd ed., Little, Brown and Company, Boston, 1960, p. 203.
3. Van den Bergh, A. and Mueller, P., Biochem. Z. 77, 1916, p. 90.
4. NCCLS: Standard Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (H3), Standard Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture (H4), Standard Procedures for Blood Specimen processing (H18), National Committee for Clinical Laboratory Standards, Villanova, PA.
5. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington, D.C.1990, p. 3-61 - 3-72.
6. Henry, R., Cannon, D.C., and Winkelman, J.W., Clinical Chemistry Principles and Technics, 2nd ed., Harper and Row, Hagerstown, 1974, p. 1042.
7. Wachtel M et al, Creation and Verifi cation of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

Order Information:

Ref./Cat. No.	Pack Size	Presentation
P-BIL(TD) - 200	2 x 100 ml	Two Reagents
PBBIL(T) - 1000	2 x 100 ml	Two Reagents

Product Features

- **Liquid stable two reagents (Total Bilirubin Reagent and Sodium Nitrite)**
- **Both Monochromatic and Bichromatic estimations.**
- **Neonatal Bilirubin can be estimated.**
- **5 minutes End Point assay.**
- **Measuring Wavelength : 546 nms (Monochromatic)**
  546 & 630 nms(Bichromatic)
- **Linearity 25 mg/dl.**
- **Serum or Heparinized Plasma as Specimens**
- **Estmination with fixed factor : Monochromatic - 25**
  Bichromatic - 27
- **Available as multipurpose reagents and dedicated system packs**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
15°C 30°C	Temperature limit	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

Pariksha Biotech
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eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
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AN ISO 13485 Certified Company

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