Pariksha Biotech
A game changer in IVD

Bilirubin (Direct) Estimation Kit

High-Q Bilirubin-(Direct)
(Jendrassik & Grof Method)

HIGH®
Command on Quality

Intented Use: Kit for quantitative the determination of of Direct Bilirubin in serum or Plasma.

Introduction:
Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. Normally it is excreted into the intestine and bile from the liver. The site of the catabolism of hemoglobin is the reticuloendothelial system (RES). Bilirubin is then released into the bloodstream where it binds tightly to albumin and is transported to the liver. Upon uptake by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide that are water soluble metabolites. The metabolites will react with aqueous diazo reagent and are commonly referred to as "direct bilirubin".

Elevation of total serum bilirubin may occur due to excessive hemolysis or destruction of the red blood cells e.g. hemolytic disease of the newborn, liver diseases e.g. hepatitis and cirrhosis obstruction of the biliary tract e.g., gallstones. There is information in the literature indicating elevated levels of direct bilirubin in patients with liver or biliary tract diseases: even though, total bilirubin levels are normal. Therefore, the greatest diagnostic value of direct bilirubin assays stem from their ability to indicate occult liver disease.

Most chemical methods for the determination of total bilirubin are based on the reaction between diazotized sulfanilic acid and bilirubin to produce azobilirubin, which absorbs maximally at 546 nm. Such tests are often run in the presence and absence of an organic solvent e.g., methanol to distinguish free bilirubin from conjugated bilirubin on a differential solubility basis.

Principle:
Direct Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 546 nm in the aqueous solution. The intensity of the color produced is directly proportional to the amount of direct bilirubin concentration present in the sample.

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 -10ºC

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:
Direct Bilirubin Reagent:

Sulphanilic Acid	:	22 mMol/L
Concentrated Hydrochloric Acid	:	120 mMol/L

Sodium Nitrite Reagent:

Sodium Nitrite:	:	100 mMol/L

System Parameters for Direct Bilirubin (Monochromatic with Sample Blank)

Type of Reaction	:	End Point
Reaction Slope	:	Increasing
Wavelength	:	546 nm
Sample Blank	:	yes
Flowcell Temperature	:	37ºC
Incubation time	:	5 min. at R.T.
Factor	**:**	**12.0 (Direct Bilirubin)**
Sample Volume	:	50µl
Reagent Volume	:	1.050 ml.
Zero setting with	:	Sample Blank

Test Procedure for Direct Bilirubin Estimation (Monochromatic Method)

Reagent	Sample Blank	Test (T)
Direct 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:

Direct Bilirubin (mg/dl)= Abs of Test-Abs of Sample Blank) x 12.0

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

Type of Reaction	:	End Point
Reaction Slope	:	Increasing
Wavelength	:	546 nm & 630
Flowcell Temperature	:	37ºC
Incubation time	:	5 min. at R.T.
Factor	**:**	**12.2 (Direct Bilirubin)**
Sample Volume	:	50µl
Reagent Volume	:	1.050 ml.
Zero setting with	:	Distilled Water

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

Reagent	Test (T)
Direct 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:

Direct Bilirubin (mg/dl)= Abs of Test x 12.2

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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:
Direct Bilirubin : Adults 0.0 – 0.32 mg/dl

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 0.998 with a regression equation of y = 1.04x + 0.07.

2. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
0.98 .	025	2.6	0.96 .	028	2.9

Bibliography:

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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.
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Ordering Information:

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

Product Features

- Liquid stable, ready to use two reagents (Direct 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 - 12.0
  Bichromatic - 12.2
- 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		

eIFU Indicator

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SCAN TO EXPLORE MORE

Manufactured in India by :
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AN ISO 13485 Certified Company

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