ESTROM Bilirubin-(Total & Direct)
(Jendrassik & Grof Method)

ANGSTROM BIOTECH PVT. LTD

Intented Use: Kit for the quantitative determination of Total and Direct Bilirubins in serum or Plasma.

PRINCIPLE:

Bilirubin is estimated by reacting it with diazotized sulfanilic acid (obtained from sodium nitrite and sulfanilic acid) forms a pink coloured azocompound. Direct Bilirubin (conjugated or soluble fraction) reacts very quickly and is read by measuring colour developed in 30 seconds or reaction. The unconjugated or free Bilirubin takes longer time to react and requires an accelerator. Hence total Bilirubin is measured using Caffeine/Dimethyl Sulfoxide as an accelerator allowing the reaction to proceed for 5 minutes.

CLINICAL SIGNIFICANCE:

Total and direct Bilirubin estimation in serum or plasma is used for the diagnosis, differentiation and follow up of jaundice.

A.Hemolytic Jaundice: Increased hemolysis results in elevation of unconjugated Bilirubin level.

B.Obstructive Jaundice: The conjugated Bilirubin increases due to regurgitation of bile into hepatic circulation, because of blockage of bile passage.

C Hepatic Jaundice: In this case increase of both conjugated and unconjugated Bilirubin serum is estimated in order to assess the extent of liver damage and subsequent progress of regress. The following table gives the findings:

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.

Reference Values:

Total Bilirubin : Adults 0.0 –1.1 mg/dl
Direct Bilirubin : Adults 0.0 – 0.32 mg/dl

Reagent Composition:

Total Bilirubin Reagent:
Sulphanilic Acid : 40 mMol/L
Concentrated Hydrochloric Acid : 200mMol/L
2,4-dichloroaniline : 0.6 mMol/L

Direct Bilirubin Reagent:
Sulphanilic Acid : 20 mMol/L
Concentrated Hydrochloric Acid : 100mMol/L

Sodium Nitrite Reagent:
Sodium Nitrite: : 280 mMol/L

Linearity :

This method is linear for Total & Direct Bilirubin Values up to 25 mg/dl

Pack Size	Presentation
2 x 100 ml	Three Reagents
4 x 50 ml	

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	:	37°C
Incubation time	:	5 min. at R.T.
Factor	:	25 (Total Bilirubin)
Sample Volume	:	50µl
Reagent Volume	:	1.050 ml.
Zero setting with	:	Distilled Water

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 Direct Bilirubin (Monochromatic with Sample Blank)

Type of Reaction	:	End Point
Reaction Slope	:	Increasing
Wavelength	:	546 nm
Sample Blank	:	yes
Flowcell Temperature	:	37°C
Incubation time	:	5 min. at R.T.
Factor	:	13.5 (Direct Bilirubin)
Sample Volume	:	50µl
Reagent Volume	:	1.050 ml.
Zero setting with	:	Distilled Water

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 13.5

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	Attention,see instructions for use		Consult Instructions For Use
IVD	For in vitro diagnostic use only	REF	Catalog #
	Store at RT	LOT	Lot Number
	Do not use if package is damaged		Date of Manufacturing
	Manufacturer		Use by