

Ana-Chek® BILIRUBIN T&D

METHOD – JENDRASSIK AND GROF

PRODUCT CODE – LB01



INSTRUCTIONS FOR USE

INTENDED USE: Test for estimation of Bilirubin Total and Direct in serum/plasma using Jendrassik & Grof method.

SUMMARY AND PRINCIPLE

Direct bilirubin is elevated in obstructive jaundice and cirrhosis of liver whereas indirect bilirubin is elevated in prehepatic disorders (haemolysis or impaired conjugation or transport) in liver. Bilirubin T & D is a reagent set for determination of Total and Direct Bilirubin based on Jendrassik and Grof method using Diazotized sulphanilic acid with DMSO as an activator for Total Bilirubin. **NO SERUM BLANK IS NEEDED FOR TEST.** Ana-Chek® Bilirubin T & D is the most suited for testing bilirubin in neonatal samples. Ana-Chek® Bilirubin T & D is a two-reagent system using one step procedure.

Bilirubin + Sulphanilic Acid + Sodium Nitrite → Purple Colour

KIT COMPONENTS

Reagent 1 : Bilirubin T1 Reagent
Reagent 2 : Bilirubin T2 Reagent
Reagent 3 : Bilirubin D1 Reagent
Reagent 4 : Bilirubin D2 Reagent

REAGENT PREPARATION, STORAGE & STABILITY

All reagents are ready to use. Working reagents prepared for test by mixing 1 ml of T1 / D1 with 20 µl T2 / D2 respectively for Total or Direct Bilirubin estimation. The working reagent is stable for 2 days at 2- 8 °C. The reagent kit should be stored at Room Temperature (<25°C) and is stable till the expiry date indicated on the label.

PRECAUTIONS & HANDLING

The reagents/samples should be handled by qualified personnel only. Discard reagent/sample as per good laboratory practices and local regulatory requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Contamination of the reagent should be avoided.

TEST PARAMETERS

Name	Bilirubin Total/Direct	Reagent Volume T1/D1	1000 µl
Reaction Type	End Point	Reagent Volume T2/D2	20 µl
Primary Wavelength	546 nm	Sample Volume	50 µl
Secondary Wavelength	600 nm	Incubation Temperature	R.T.
Flow Cell Temp.	37 °C	Incubation Time	5 min
Blank setting	D.W.	Factor	15 – Direct Bilirubin
Linearity	20 mg/dL		30 – Total Bilirubin

MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes, Micropipette with tips, Analyzer, Controls, Incubation chamber.

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Serum is preferred. Bilirubin is light sensitive. Avoid exposure of sample to direct light. Bilirubin in sample is stable for 1 day at 2° - 8° C and one month at - 10° C.

COMPONENTS OF REAGENT

Component	Direct Bilirubin	Total Bilirubin
Sulphanilic Acid	5 mmol/L	5 mmol/L
Sodium Nitrite	144 mmol/L	72 mmol/L
Hydrochloric Acid	165 mmol/L	165 mmol/L
DMSO	-	7 mmol/L
Stabilizers, inactive ingredients and surface-active agents.		

ASSAY PROCEDURE

	Bilirubin Total	Bilirubin Direct
Reagent T1	1000 µl	-
Reagent T2	20 µl	-
Reagent D1	-	1000 µl
Reagent D2	-	20 µl
Serum / Plasma	50 µl	50 µl
Mix the reagent and sample in the above-mentioned ratio and incubate for 5 mins at R.T.		
Aspirate reaction mixture into flow cell and record the absorbance.		
Final colour is stable for 8 mins if not exposed to direct light.		

CALCULATION

Direct Bilirubin (mg/dL) = Absorbance of sample x 15
Total Bilirubin (mg/dL) = Absorbance of sample x 30

REFERENCE VALUES FOR NORMAL PEOPLE

Total Bilirubin – upto 1.2 mg/dL
Direct Bilirubin – upto 0.5 mg/dL

PERFORMANCE CHARACTERISTICS

Measuring Range: The assay is linear between 0.150 - 20 mg/dL. If the Bilirubin value exceeds linearity limit (above 20 mg/dL), dilute the specimen suitably with normal saline and repeat the assay. In that case, assay value should be multiplied with the dilution factor to obtain correct bilirubin value of the specimen.

Interference: There is no significant interference in samples containing Triglycerides upto 1000 mg/dL. Haemolysis causes decreased Bilirubin values.

Precision: Precision studies has been carried out using quality control sera as shown below:

Bilirubin Total

(n=10)	Within Run			Between Run		
Specimen Material	Mean (mg/dL)	SD (mg/dL)	CV %	Mean (mg/dL)	SD (mg/dL)	CV %
Low Value Serum	0.98	0.01	1.2	0.86	0.01	1.6
High Value Serum	4.25	0.06	1.5	3.65	0.07	1.9

Bilirubin Direct

(n=10)	Within Run			Between Run		
Specimen Material	Mean (mg/dL)	SD (mg/dL)	CV %	Mean (mg/dL)	SD (mg/dL)	CV %
Low Value Serum	0.29	0.00	0.6	0.26	0.00	1.7
High Value Serum	1.13	0.04	3.1	0.94	0.02	2.3

Note: We recommend all laboratories to establish their own accuracy and precision data.

QUALITY CONTROL

Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for Bilirubin. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

PRECAUTIONS

1. If Bilirubin value exceeds 20 mg/dL then dilute the specimen suitably with normal saline & repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain the correct bilirubin value.
2. Gross haemolysis may cause falsely decreased results in bilirubin assays and should be avoided.
3. Gross lipaemia may cause falsely elevated results unless a sample blank is used.

BIBLIOGRAPHY

1. Jendrassik L.Grof P.Biochem 2.297,81(1938)
2. Practical Clinical Biochem.vol 1,5th edition, H.varley, page 1012, (1980).
3. Inhouse test data.

Symbol	Explanation	Symbol	Explanation
	Manufactured By		In Vitro Diagnostic Use
	Lot Number		Read Instructions Before Use
	Catalogue Number		Storage Temperature
	Manufacturing Date		Number of Tests / Volume
	Expiry Date		Do Not Reuse
	Protect from Sunlight		Keep Dry

ANAMOL LABORATORIES PVT. LTD.
61, Genesis Industrial Township, Kolgaon,
Palghar – 401 404. India.
Customer Care & WhatsApp: +91-9823388695.

admin@anamollabs.com
exports@anamollabs.com
www.anamollabs.com

ISO 9001: 2015
ISO 13485: 2016
GMP
CE