

# BILIRUBIN

## (Jendrassik & Grof Method)

Reagent kit for quantitative estimation of Total and Direct Bilirubin in serum or plasma.

### 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 in serum is estimated in order to assess the extent of liver damage and subsequent progress or regress.

The following table gives the findings.

	HEMOLYTIC	HEPATIC	OBSTRUCTIVE
DIRECT/SOLUBLE BILIRUBIN	NORMAL	INCREASED	INCREASED
INDIRECT OR FREE BILIRUBIN	INCREASED	INCREASED	NORMAL

### 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 bilirubin are taken after 5 mins. incubation & the readings for direct Bilirubin are taken after 3 mins. incubation.

### SAMPLE COLLECTION, STORAGE & STABILITY:

Serum is preferred, plasma with heparin as anticoagulant may be used. Serum or plasma should be separated as early as possible. Sample are stable for a day when stored tightly capped at 2-8°C and for a month at -10°C

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 hemolysed or cross contaminated samples.

### REAGENT COMPOSITION :

Active Ingredients	Concentration
<b>Regent-1 A</b>	
* Buffer	100 mmol/L
* Sodium Nitrite	10 mmol/L
pH 7.0 ± 0.1 at 250°C	
<b>Regent-1 B</b>	
* Sulphanilic Acid	20 mmol/L
<b>Regent-2</b>	
* Sodium Chloride	100 mmol/L
<b>Regent-3</b>	
* Sodium Benzoate	130 mmol/L
* Caffeine	100 mmol/L

Also contains non-reactive fillers and Stabilizers.

### PRESENTATION:

	No of bottle / Vial	
	2x30ml	3x60ml
All the reagents to be stored at 2-8°C		
1 - BILIRUBIN (A)	1	3
1- BILIRUBIN (B)	1	1
2 - BILIRUBIN	1	1
3 - BILIRUBIN	2	3

### PRECAUTIONS:

BILIRUBIN IS FOR *IN-VITRO* diagnostic use only  
Reagent contains Sodium Azide, DO NOT INGEST.

### STORAGE & STABILITY OF THE REAGENTS:

All the reagents in the kit are stable at 2-8°C until expiry date stated on the labels. **The 1-Bilirubin A solution is stable for 3 months at 2-8°C after opening.**

### PREPARATION OF WORKING SOLUTIONS:

Allow the reagents to attain the room temperature. The working solutions should be prepared fresh according to the need.

### WORKING SOLUTION -1

Mix equal volume of 1-Bilirubin reagent A & 1-Bilirubin reagent B.

### SOLUTION - 2

2- Bilirubin reagent is ready to use.

### SOLUTION -3

3-Bilirubin reagent is ready to use.  
(Bring the 3-Bilirubin reagent to R.T. before use.)

### NOTE:

Please note that Reagent - 3 may develop needle shaped crystals at low temperature which should be dissolved by warming at 37°C before use.

### PROCEDURE:

The sample should be brought to room temperature before use. The following reaction parameters are to be used with this kit.

### REACTION PARAMETERS FOR TOTAL BILIRUBIN:

<b>Monochromatic</b>	
Type of Reaction	: End Point
Wavelength	: 546 nm (530-560 nm)
Flowcell Temperature	: 30°C
Incubation time	: 5 min. at R.T. (25°C ± 5°C)
Factor	: 26.312
Sample Volume	: 50µl
Reagent Volume	: 1.1 ml.
Zero setting with	: Distilled water
<b>Bichromatic</b>	
Other parameter as above	
Wavelength	: 546 nm and 630 nm
Sample Blank	: No
Set the instrument using above system parameters	



**REACTION PARAMETERS FOR DIRECT BILIRUBIN :**

**Monochromatic**

Type of Reaction	:	End Point
Wavelength	:	546 nm (530-560 nm)
Flowcell Temperature	:	30°C
Incubation time	:	3 min. at R.T. (25°C ± 5°C)
Factor	:	26.312
Sample Volume	:	50 µl
Reagent Volume	:	1.1 ml
Zero setting with	:	Distilled Water

**Bichromatic**

Other parameter as above	:	
Wavelength	:	546 nm and 630 nm
Sample Blank	:	No

Set the instrument using above system parameters

**PROCEDURE FOR TOTAL BILIRUBIN :**

**A) MONOCHROMATIC METHOD :**

	Sample Blank	Test
Sample	50 µl	50 µl
Working Solution 1	-	100 µl
Solution 2	100 µl	-
Solution 3	1.0 ml	1.0 ml

Mix & incubate for 5 mins. at R.T. & read the absorbance against sample blank at 546 nm. Read the result before 10 mins.

**B) BICHROMATIC METHOD:**

	Test
Sample	50 µl
Working Solution 1	100 µl
Solution 3	1.0 ml

Mix & incubate for 5 mins. at R.T. & Read the results before 10 mins.

**PROCEDURE FOR DIRECT BILIRUBIN :**

**A) MONOCHROMATIC METHOD :**

	Sample Blank	Test
Sample	50 µl	50 µl
Normal Saline	1.0 ml	1.0 ml
Working Solution-1	-	100 µl
Solution 2	100 µl	-

Mix & incubate for 3 mins. at R.T. & read the absorbance against sample blank at 546 nm.

**B) BICHROMATIC METHOD:**

	Test
Sample	50 µl
Normal Saline	1.0 ml
Working Solution 1	100 µl

Mix & incubate for 3 mins. at R.T. & Read the results before 10 mins.

**CALCULATION:**

Serum Bilirubin (mg/dl)=(Abs. of sample-Abs. of sample blank) X F

**LIMITATIONS FOR INTERFERENCE:**

As per studies carried out for interference. Following results were obtained.

- No Interference from Hemoglobin upto 50 mg/dl.

**NORMAL VALUES :**

- TOTAL BILIRUBIN : 0-1.0 mg/dl
- DIRECT BILIRUBIN : 0-0.3 mg/dl

**LINEARITY:**

> 15 mg/dl.

**REFERENCE:**

- JENDRASSIK L & GROF P (1938) BIOCHEM.Z. 297. 81-89.

