

BILIRUBIN KIT

(Mod. Jendrassik & Grof's method)
For the determination of Direct & Total Bilirubin in serum
(For In vitro Diagnostic Use Only)

CLINICAL SIGNIFICANCE

Bilirubin is mainly formed from the heme portion of aged or damaged RBC's. It then combines with albumin to form a complex, which is not water soluble. This is referred to as indirect or unconjugated Bilirubin. In the liver this Bilirubin complex is combined with glucuronic acid into a water soluble conjugate. This is referred to as conjugated or direct Bilirubin.

INCREASES

Elevated levels of bilirubin are found in liver diseases (Hepatitis, cirrhosis), excessive haemolysis/destruction of RBC (hemolytic jaundice) obstruction of the biliary tract (obstructive Jaundice) and in drug induced reactions.

The differentiation between the direct and indirect bilirubin is important in diagnosing the cause of hyperbilirubinemia.

METHODOLOGY : Mod. Jendrassik & Grof's method

PRINCIPLE

Bilirubin + Diazotized Sulphanilic acid → Azobilirubin Compound

REAGENT COMPOSITION

TOTAL BILIRUBIN		DIRECT BILIRUBIN	
Sulphanilic acid	- 10 mmol/L	Sulphanilic acid	- 10 mmol/L
Conc. HCl	- 40 mmol/L	Conc. HCl	- 40 mmol/L
Sodium Nitrite	- 15 mmol/L	Sodium Nitrite	- 1.5 mmol/L
Caffeine	- 25 mmol/L		
Sodium Benzoate	- 20 mmol/L		

STORAGE AND STABILITY

All reagents are stable at R.T., till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents are ready to use. **Do not pipette with mouth.**

SAMPLE MATERIAL

Serum Bilirubin is reported to be stable in the sample for 4 days at 2-8°C protected from light as it is photosensitive.

ASSAY PARAMETERS

Reaction	End Point	Interval	-
Wavelength	546 nm	Sample Vol.	0.05 ml
Zero Settings	Sample Blank	Reagent Vol.	1.025 ml
Incub. Temp	R.T / 37°C	Standard	-
Incub Time	10 min /5 min	Factor	26.30
Delay Time	-	React. Slope	Increasing
Read Time	-	Linearity	25 mg/dl
No. of read.	-	Units	mg/dl

ASSAY PROCEDURE

Wavelength / filter : 546 nm / Yellow – Green
Temperature : R.T
Light path : 1 cm

DIRECT BILIRUBIN ASSAY

Pipette into clean dry test tubes labelled as Blank (B) and Test (T):

Addition Sequence	B _D (ml)	T (ml)
Direct Reagent (A ₁)	1.0	1.0
Direct Nitrite Reagent (A ₂)	-	0.025
Mix well and proceed	-	-
Sample	0.05	0.05

Mix well and incubate at R.T. for exactly 10 min or 5 min at 37°C. Measure the absorbance of the Test samples (Abs.T) immediately against their respective Blanks.

TOTAL BILIRUBIN ASSAY

Pipette into clean dry test tubes labelled as Blank (B), and Test (T):

Addition Sequence	B _T (ml)	T (ml)
Total Reagent (A ₁)	1.0	1.0
Total Nitrite Reagent (A ₁₁)	-	0.025
Mix well and proceed	-	-
Sample	0.05	0.05

Mix well and incubate at R.T. for exactly 10 min or 5 min at 37°C. Measure the absorbance of the Test samples (Abs.T) immediately against their respective Blanks.

CALCULATIONS

Direct Bilirubin in mg/dl = (Abs. T – Abs.B_D) x 26.30 (factor)

Total Bilirubin in mg/dl = (Abs. T – Abs.B_T) x 26.30 (factor)

LINEARITY

This procedure is linear upto 25 mg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

NOTE

- In case of cuvette Volume is more than 1.0 ml requisite volume of reagents and sample can be multiplied keeping reagent to sample ratio same.
- Sequence of reagent addition should be followed strictly as per the procedure.

QUALITY CONTROL

To ensure adequate quality control each run should include assayed normal and abnormal controls.

NORMAL REFERENCE VALUES

Serum (Direct) : upto 0.2 mg/dl

(Total) : upto 1.0 mg/dl

It is recommended that each laboratory establish its own normal range representing its patient population.

REFERENCE :

- Jendrassik L and Grf.P. (1938) BIOCHEM. 2;297.81.

PRESENTATION DIRECT BILIRUBIN

PRODUCT CODE	PACK SIZE	DIRECT REAGENT(A ₁)	DIRECT NITRITE REAGENT (A ₁₁)
ABL 0604	50+50T	1 X 100 ml	1x 5 ml

TOTAL BILIRUBIN

PRODUCT CODE	PACK SIZE	TOTAL REAGENT(A ₁)	TOTALNITRITE REAGENT (A ₁₁)
ABL 0604	50+50T	1 X 100 ml	1x 5 ml

PRODUCT FEATURES AT A GLANCE :

- Simple 2 Reagent system 1 ml procedure.
- Ready to use Reagent, No dilution steps, avoid contamination.
- Combi Pack, Direct & Total Bilirubin estimation in a single kit.
- Common factor for Total & Direct Bilirubin.
- Highest linearity 25 mg/dL.
- Shelf life 24 Months.
- Can be tested in Analyzers and Colorimeters.
- Convenient pack size – 50 + 50 T.
- Store at Room temperature.



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IN VITRO DIAGNOSTIC REAGENTS

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