Bilirubin Direct

(Jendrassik and Grof Method)

Intended Use

Bilirubin Direct is a reagent kit used for the determination of direct Bilirubin based on Jendrassik and Grof method.

Principle

Bilirubin reacts with diazotized Sulphanilic acid to produce azobilirubin (Pink colour). The pink colour thus produced is proportional to Direct Bilirubin concentration measured at 546/630nm .

Bilirubin + Sulphanilic acid + Sodium nitrite ⟶ Azobilirubin

Reagents provided

1. Bilirubin Direct Reagent (R1) - Ready to use
2. Bilirubin Direct Reagent (R2) - Ready to use

Reagent storage and stability

The reagent kit should be stored at Room Temperature and is stable till the expiry date indicated on the label.

Specimen collection and preservation

Blood should be collected in a clean dry container. Although serum is preferred, plasma can also be used as sample. Following anticoagulants can be used for plasma separation:

- EDTA 2 mg/ml of blood
- HEPARIN 200 IU/ml of blood

Bilirubin is light sensitive. Avoid exposure of serum or plasma to direct light. Bilirubin in serum and plasma is stable for a day at 2° - 8°C or one month at -20°C. Samples should be brought to room temperature before use.

Assay guidelines for Analyzers

Reaction Type	End Point
Reaction slope	Increasing
Incubation time	5 min. at 37°C
Wave length	546 / 630 nm
Blank with	Distilled Water
Sample Volume	50 µl (0.05 ml)
Reagent Volume R1	800 µl (0.8 ml)
Reagent Volume R2	200 µl (0.2 ml)
Factor	17
Low normal	0 mg/dl
High normal	0.42 mg/dl
Linearity	Up to 30 mg/dl

Assay guidelines for Manual Procedure

Note: The reagents R1, R2 and sample require proper mixing

Reagents	**Test**
Reagent R1	800 µl (0.8 ml)
Reagent R2	200 µl (0.2 ml)
Serum / Plasma	0.05 ml

1) Mix thoroughly and incubate at 37°Cfor 5 minutes.
2) The absorbance of the test against distilled water at 546/630nm.

Calculation

Con. in sample mg/dl = Abs. of Test x 17

Normal range

Direct Bilirubin : Up to 0.42 mg/dl

Note: Expected range varies from population to population and each laboratory should establish the normal range for its own population.

Limitations

KEE DIAGNOSTICS

Dilute the specimen if the Bilirubin value is above 30 mg/dl. Suitable dilution can be done with normal saline. In such case the results obtained should be multiplied by dilution factor to obtain correct Bilirubin value.

Quality Control

It is recommended that each batch should include a normal and an abnormal commercial reference control serum or a known patient serum. Use of quality control serum, checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, accuracy of pipetting and serum exposure to light.

References

1. Jendrassik, L., et al.(Biochem. 2. 297, 81 (1938)
2. Practical Clinical Biochem. Vol 1, 5th edition, H. Varley, page 1012, (1980).

KEE DIAGNOSTICS

Symbols

IVD	In Vitro Diagnostics.		Caution.		Keep away from sun light.		Date of Manufacture.
LOT	Batch No.		Read Instructions.		Fragile.		Product Expiry Date.
CONT	Content.		Storage Temperature.		Keep Dry.		Manufactured By.
REF	Catalogue No.						

KEE DIAGNOSTICS PVT LTD
CIN: U24231DL2004PTC128343 .
A subsidiary of KEE PHARMA LTD
A-8 . 3RD FLOOR. NARAINA INDUSTRIAL AREA, PHASE 2.
NEW DELHI-110028.
W. www.keediagnostics.com.
E. info@keediagnostics.com, T. 011 43136000.

ISO 9001 : 2015 & ISO 13485 : 2016 Certified Company