

ANA BIO CLR Bilirubin Direct

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 nm (530 - 550 nm).

Bilirubin + Sulphanilic acid + Sodium nitrite \longrightarrow 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 2° - 8°C and is stable till the expiry date indicated on the label.
The working solution is stable for 2 days at 2° - 8°C and for 5 hours at room temperature (25° - 30°C).

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	Deferential
Reaction slope	Increasing	Increasing
Incubation time	5 min. at RT (25° - 30 °C)	5 min. at RT (25° - 30 °C)
Wave length	546 nm (530 - 550 nm)	546 / 670
Blank	Serum Blank	Dis. water
Sample Volume	50 µl (0.05 ml)	50 µl (0.05 ml)
Reagent Volume R1	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Reagent Volume R2	20 µl (0.02 ml)	20 µl (0.02 ml)
Factor	10	10
Low normal	0 mg/dl	0 mg/dl
High normal	0.3 mg/dl	0.3 mg/dl
Linearity	Up to 30 mg/dl	Up to 30 mg/dl

Assay guidelines for Manual Procedure

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

Reagents	Serum blank	Test
Reagent R1	1.0 ml	1.0 ml
Reagent R2	-	0.02 ml
Serum / Plasma	0.05 ml	0.05 ml

- 1) Mix thoroughly and incubate at room temperature (25° - 30°C) for 5 minutes.
- 2) Read before 8 minutes the absorbance of the test against their respective blanks at 546 nm (530-550 nm).
(OR)
- 3) Read Before 8 min. without sample blanks in Deferential mode at 546/670 nm

Calculation

Con. in sample mg/dl = (Abs. of Test – Abs. of Serum Blank) x 10

Normal range

Direct Bilirubin : Up to 0.3 mg/dl

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

Limitations

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).

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.						



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