

Direct BILIRUBIN liquid

Modified Jendrassik method

R1: 1 x 100 ml + R2: 1 x 10 ml
R1: 3 x 100 ml + R2: 1 x 30 ml

CL08-110
CL08-330

INTENDED USE

Kit for quantitative determination of Direct Bilirubin in serum.

CLINICAL MEANING

Analysis of total and direct bilirubin is made to determine the presence of hepatic damages or diseases e.g. obstruction of bile ducts, haemolytic anemias, metabolic issues, stones. A typical sign of high bilirubin levels is jaundice, which manifests with yellow skin and yellow sclerae. An increase in direct bilirubin is a symptom of a hepatic problem, especially of the biliary excretion tract.

PRINCIPLE

In an acid medium, direct bilirubin reacts with diazotized sulphanilic acid to form a pink diazo compound (azobilirubin), whose intensity is proportional to the concentration of direct bilirubin present in the sample.

SAMPLE

Non hemolyzed serum.

Analyze samples within 2 hours after collection. Protect samples from light.

STABILITY: 12 hours in the fridge at 2-8°C, 3 months at -20°C if protected from light.

REAGENTS

Only for in Vitro diagnostic use. Liquid mono-reagent ready to use.

| Package content | CL08-110 | CL08-330 |
|--|------------|------------|
| REAGENT 1 Sulphanilic acid 1,75 mmol/L, hydrochloric acid 0,015 mmol/L | 1 x 100 ml | 3 x 100 ml |
| REAGENT 2 Sodium nitrite 7 mmol/L | 3 x 10 ml | 1 x 30 ml |

Stability: Store at 15-30°C and protect from light to keep the reagents stable up to the expiration date on the label. Keep bottles closed when not in use. Do not use turbid reagents.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

MANUAL ASSAY PROCEDURE

| | |
|-----------------------|----------------------|
| Wavelength: | 546 nm |
| Optical Path: | 1 cm |
| Reading: | Against blank sample |
| Temperature: | 37 °C |
| Method: | Increasing endpoint |
| Reaction time: | 10 minutes |
| Sample/reagent Ratio: | 1/16 |

Bring reagents to the chosen temperature for the analysis.

Pipette in cuvette:

| | Blank sample | Sample |
|----------------------|--------------|--------|
| Reagent 1 | 1,5 ml | 1,5 ml |
| Reagent 2 | - - - | 100 µl |
| De-mineralized water | 100 µl | - - - |
| Sample | 100 µl | 100 µl |

Stir carefully. After exactly 10 minutes of incubation at 37°C, read the sample absorbance (AbsS) against the blank sample (AbsSB). The color is stable for about 60 minutes at room temperature and protected from direct light.

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

Calculate the concentration in the sample using the following formula:

$$[\text{mg/dl}] \text{ direct bilirubin} = (\text{AbsS} - \text{AbsSB}) \times 14,5$$

$$[\mu\text{mol/l}] \text{ direct bilirubin} = (\text{AbsS} - \text{AbsSB}) \times 239$$

REFERENCE VALUES

Up to 0,25 mg/dl (4,3 µmol/L).

Each laboratory should define its own reference values for this method.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program.

Control serums of human origin are available for this purpose on request:

PRE-NORM serums with normal values

PRE-PATH serums with pathological values

If the method requires it, a multi-parameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is 0,05 mg/dl.

Linearity: up to 25 mg/dl (427 µmol/L).

For higher values, dilute the samples with saline solution and multiply the result by the dilution factor.

Precision:

| Within run (n=10) | Average [mg/dl] | SD | CV % |
|-------------------|-----------------|-------|------|
| Sample 1 | 0,6 | 0,009 | 1,50 |
| Sample 2 | 2,30 | 0,08 | 3,48 |

| Between run (n=20) | Average [mg/dl] | SD | CV % |
|--------------------|-----------------|-------|------|
| Sample 1 | 0,6 | 0,015 | 2,50 |
| Sample 2 | 2,38 | 0,101 | 4,24 |

Interferences: up to 150 mg/dl of hemoglobin does not interfere.

Direct light can cause a decrease of direct bilirubin up to 50% in an hour.

Correlation against a reference method: the correlation of FAR method (Y) against a reference method (X) gives this equation:

$$Y = 1,0453X + 0,0062$$

$$r = 0,9844$$

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

The reagents may contain non-reactive components and various preservatives. Contact with the skin and ingestion should be avoided. Use the normal precautions expected with correct behaviour in laboratory.

REFERENCES

1. Pearlman F.C., Lee R.T.Y., Clin. Chem. 20, 447, (1974)
2. Blumenfeld T.A. et al., Am. J. Clin. Path. 69, 388 (1978)

MANUFACTURER

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KEY SYMBOLS

| | |
|---|------------------------------------|
|  | In Vitro diagnostic medical device |
|  | batch number |
|  | catalog number |
|  | temperature limits |
|  | use by |
|  | caution |
|  | consult accompanying documents |

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