# Direct BILIRUBIN

DPD Colorimetric method

R1: 2 x 100 ml + R2: 2 x 25 ml CL09-250

## 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 amenias, 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, total bilirubin reacts with 3.5-DPD forming a red-colored diazo compound (azobilirubin), whose intensity is proportional to the concentration of direct bilirubin present in the sample.
While direct bilirubin, that is conjugated with glucuronic acid, is water-soluble and reacts directly, total bilirubin is obtained through the presence of an accelerator that splits the bond with albumin.
Indirect bilirubin can be calculated by the difference between total bilirubin and direct bilirubin.

## 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** | | **CL09-250** |
|---|---|---|
| **REAGENT 1**<br>Hydrochloric acid 170 mM | | **CL09-250R1**<br>2 x 100 ml |
| **REAGENT 2**<br>3,5-DPD 0,5 mM<br>Hydrochloric acid 170 mM | | **CL09-250R2**<br>2 x 25 ml |

Stability: Store at 2-8°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

| | |
|---|---|
| Primary wavelength: | 546 nm |
| Reference wavelength: | 800 nm |
| Optical Path: | 1 cm |
| Reading: | Against blank sample |
| Temperature: | 37 °C |
| Method: | Increasing endpoint |
| Reaction: | two final points |
| Ratio: | 2/20/5 |

Bring reagents to the chosen temperature for the analysis.

Pipette in cuvette:

Pipettare in cuvetta:

| | Calibrator | Sample |
|---|---|---|
| Reagent 1 | 1 ml | 1 ml |
| Sample | --- | 100 µl |
| Calibrator | 100 µl | --- |

Stir carefully. After exactly 5 minutes of incubation at 37°C, read the Blank Calibrator (AbsBCal) and the blank sample (AbsBS) against water.

Add:

| | | |
|---|---|---|
| Reagent 2 | 250 µl | 250 µl |

Stir carefully. After exactly 5 minutes of incubation at 37°C, read the Calibrator (AbsCal) and the Sample (AbsS) against water

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

## CALCULATION

Calculate the concentration in the sample using the following formula:

$$\frac{AbsS - AbsBS}{AbsCal - AbsBCal} \times Val.\ Cal = BIL\text{-}D\ (mg/dl)$$

## REFERENCE VALUES

Up to 0.35 mg/dl ( 5.1µmol/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 (REF 7532).

## PERFORMANCE CHARACTERISTICS

**Sensitivity:** the sensitivity of the method is 0.06 mg/dl.

**Linearity:** up to 20 mg/dl.
For higher values, dilute the samples with saline solution and multiply the result by the dilution factor.

**Precision:**

| Within run (n=30) | Average [mg/dl] | CV % |
|---|---|---|
| Sample 1 | 0.21 | 2.8 |
| Sample 2 | 3.12 | 0.9 |

| Between run (n=20) | Average [mg/dl] | CV % |
|---|---|---|
| Sample 1 | 0.24 | 3.12 |
| Sample 2 | 4.8 | 1.4 |

**Interferences:**

- up to 500 mg/dl of hemoglobin does not interfere.
- up to 1000 mg/dl triglycerides do 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 = 0.991x – 0.10 0 (n=30) r = 0.997 (n=30)

## 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

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

Issue 01 - Jan 2021 RR