

Reagent kit for the quantitative determination of direct bilirubin in serum. DPD method.

Principle

Indirect bilirubin is liberated by the detergent. Total bilirubin is coupled with the 3,5-dichlorophenyl-diazonium-tetrafluoroborate (DPD) to yield the corresponding azobilirubin. The absorbance of this dye at 546 nm is directly proportional to the total bilirubin concentration in the sample.

Reference value

Serum: total bilirubin <17,0 μmol/l (<10mg/l)

It is recommended that each laboratory should assign its own normal range.

Reagents

Total Bilirubin

1. Reagent (R1)	
Caffeine	30 mmol/l
Detergent	
Preservative	
2. Reagent (R2)	
Hydrochloric acid	170 mmol/l
DPD	3 mmol/l
Preservative	

Safety instructions:

Reagent 1:

X, Irritant
R36 Irritating to eyes
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Reagent 2:

X, Irritant
R36 Irritating to eyes
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Sample

Serum free of haemolysis or EDTA, citrate plasma. Heparin plasma not recommended.
Bilirubin in serum is light sensitive and it is recommended that serum be stored in the dark.

Procedure

All reagents are ready for use.

Avoid direct exposure to light!

Assay Conditions

Wavelength:	550 nm (540-560 nm)
Temperature:	37 °C
Cuvette:	1 cm light path
Measure:	end point
Read against:	reagent blank

Pipette into cuvette

	Sample	Calibrator
Reagent 1	1000 μl	1000 μl
Sample	40 μl	-
Calibrator	-	40 μl

Mix and incubate for 3 minutes, read the absorbance (A1).

	Sample	Calibrator
Reagent 2	250 μl	250 μl

Mix and incubate for 5 minutes, read absorbance (A2). ΔA=A2-A1

Calibration: (37°C, DPD)

S1: Distilled water
S2: Diagnosticum DunaCal or Roche C.F.A.S. (Calibrator for automated system)
Calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Calibrator}}} \times C_{\text{Calibrator}} = C_{\text{Sample}}$$

A = Absorbance, C = Concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using Cobas Mira Plus analyzer

Linearity

The test is linear up to 670 μmol/l (39 mg/dl)

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual condition the change of 0,001 absorbance is equivalent to 2 μmol/l (0,1 mg/dl) bilirubin concentration

Precision

	Reproducibility		
	average concentration (μmol/l)	SD	CV%
sample 1	17,05	0,77	4,5
sample 2	90,02	1,23	1,37

	Repeatability		
	average concentration (μmol/l)	SD	CV%
sample 1	17,03	0,88	5,03
sample 2	91,3	1,75	2,08

Correlation

Comparative studies were done to compare our reagent with another commercial bilirubin total test.

The results are detailed below:

Correlation coefficient: r=0,9803

Linear regression: y=0,975x+3,4 (x=other commercial reagent, y=own reagent)

Specificity

Ascorbic acid 2,5 mg/dl, hemoglobin 60 mg/dl don't interfere with the assay up to the given levels.

Note

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solution and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only!

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation.

 Batch Code

 Code

Bibliography

Tietz N.W., *Clinical guide to laboratory tests*, Saunders Co.
Thomas L., *Clinical Laboratory Diagnostics*, TH-Books (1998)