BILIRUBIN DIRECT

Diagnostic reagent for determination of Bilirubin direct concentration.

Liquid. Dual Reagents. Store at 2°C - 8°C. For in Vitro Diagnostic Use. Do not freeze

Ref No	Pack	Ref No	Pack	Ref No	Pack	Ref No	Pack
A2050	5 x 100 mL	DM2050	720 Tests	BY2050	6136 Tests	K2051	3636 Tests
A2051	5 x 50 mL	R2050	2885 Tests	BY2051	4091 Tests	M2050	2512 Tests
A2052	5 x 25 mL	R2051	962 Tests	N2050	800 Tests	M2051	1675 Tests
T2050	3000 Tests	S2050	2609 Tests	N2051	400 Tests	L2050	3750 Tests
T2051	1556 Tests	S2051	1565 Tests	K2050	5455 Tests	L2051	2000 Tests

INTENDED USE

The test is applied for the quantitative determination of bilirubin direct in serum.

TEST PRINCIPLE

Conjugated (direct) bilirubin reacts with diazotized 2,4 - dichloroaniline in acidic solution to produce an intensely coloured red diazo compound (520-560 nm). The intensity of color of this dye in solution is proportional to the concentration of direct bilirubin.

TEST PARAMETERS

Method	: Colorimetric, Increasing Reaction, Endpoint (Sample Blank)
Wavelength	: 546 nm
Temperature	: Room temperature, 37°C
Sample	: Non-hemolyzed serum or plasma, (store light-protected)
Linearity	: 0.2 mg/dL - 13 mg/dL

REAGENT COMPOSITION

Components	Final Concentration
Reagent 1	
Sodium chloride	≤ 0.01 M
EDTA	≤ 0.30 M
Reagent 2	
Diazotized 2,4-dichloroaniline	≤ 0.12 mM
Hydrochloric acid	≤ 0.22 M
EDTA	≤ 0.01 M

REAGENT PREPARATION

Use separate reagent ready to use.
Avoid from light sources.
Caution: keep well refrigerated.
Working reagents are stable at 2-8ºC in case of closed vials and avoiding contamination after preparation.

For manual working procedures; if working reagent will be used; shake the Reagent 2 vial gently before pouring its contents into the Reagent 1 bottle. It is advisable to wash the Reagent 2 vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.

REAGENT STABILITY AND STORAGE

Reagens are stable up to expiration date on labels at 2-8°C.
Once opened vials are stable minimum 60 days at 2-8°C at optimum conditions. On board stability is strongly related to auto analyzers cooling specification and carry-over values.

SAMPLE

Samples are collected by standard procedures.
Bilirubin direct in serum is stable for 2 days at 20 - 25°C, 7 days at 2-8ºC and 3 months at -20°C.

Use only clear, unhemolyzed serum. Bilirubin is unstable in the samples and the assay should be completed within 2 hours from collection. If longer delay is unavoidable, refrigerate the samples.

Protect samples from direct solar and artificial light

TEST PROCEDURE

Sample Start
There have many ready application procedures dedicated to different kind of photometers and ready manual working process can be supplied on request.
There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

Substrate Start
There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

CALCULATION

For the calculation of Bilirubin Direct in mg/dL sample, perform Bilirubin Direct assay and calculate as follows:

Direct Bilirubin Concentration = F x ΔA (S-SB)

Unit Conversion

mg/dL x 17.1 = µmol/L

REFERENCE INTERVAL (NORMAL VALUES) (Based on CLSI C28-P Document)*

Adults: ≤ 0.20 mg/dl (≤ 3.4 µmol/L)

*It is recommended that each laboratory establish its own reference range.

QUALITY CONTROL AND CALIBRATION

Commercially available control material with established values determined by this method may be used.
We recommend:

"ARCON N", Assayed Control Serum Normal
Cat.No. A3910

"ARCON P", Assayed Control Serum Abnormal
Cat.No. A3920

The assay requires the use of a Bilirubin Direct Standard or a Bilirubin Calibrator. We recommend:

ARCAL Calibrator ("Arcal Auto")
Cat. No. A39050

Any commercially available Standard or Calibrator suitable for this method may be used.

*Calibration Stability: It is strongly depend of application to auto analyzers and auto analyzers specification. Calibration stability is 30 days.

*Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Quality control is recommended every morning. Calibration is not recommended if QC control values are acceptable. Reagent should be calibrated after lot changes.

PERFORMANCE CHARACTERISTICS

Low linearity: 0.2 mg/dL.

High Linearity: The assay is linear up to 13 mg/dL. Samples with bilirubin concentrations higher than 13 mg/dL should be diluted with distilled or deionized water and the assay should be repeated; multiply results by dilution factor.

Linearity may considerably vary depending on the instrument used.

Precision Studies (Based on CLSI EP5 Doc.):

Repeatibility (within run) (Intra-assay)

Mean conc.	SD	CV	n
0.734 mg/dL	0.005	0.70%	10
2.488 mg/dL	0.015	0.60%	10

Reproducibility (run to run) (Inter-assay)

Mean conc.	SD	CV	n
0.898 mg/dL	0.023	2.60%	20
2.355 mg/dL	0.065	2.80%	20

Sensitivity (LOD) (Based on CLSI EP17 document): The limit of detection is 0.12 mg/dL

Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

These performance characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure is used.

NOTES

1. For in vitro diagnostic use only. Do not pipette by mouth. Avoid contact with skin and mucous membranes.
2. All the calibrators, controls and some reagents must be considered as human & animal sample, so potentially infectious; all the protection actions must be applied to avoid any potential biological risk.
3. Material safety data sheet will be supplied on request.
4. Exercise the normal precautions required for handling laboratory reagents.
5. After measurements are taken, reagent bottles should cap and kept at 2-8°C. Caps of the reagents bottles cannot be used between two different kind of reagent and between R1 & R2.
6. Reagents with different lot numbers should not be interchanged or mixed.
7. The linearity limit depends on the sample to reagent ratio.

PRECAUTIONS AND WASTE DISPOSAL

This product is made to be used in professional laboratories and by professional operators. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.
R36/38 : Irritating to eyes and skin.
S20/21 : When using, do not eat, drink or smoke.
S26 : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 : After contact with skin wash immediately with plenty of water.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45 : In case of accident or if you feel unwell, seek medical advice immediately.

S56 : Dispose of this material and its container at hazardous or special waste collection point.
S57 : Use appropriate container to avoid environmental contamination.
S61 : Avoid release in environment. Refer to special instructions/safety data sheets.
Please consult local regulations for a correct waste disposal.

ABBREVIATIONS

CLSI : Clinical and Laboratory Standards Institute
CV% : Coefficient of Variation Percentage
EP : Evaluation Protocols
GLP : Good Laboratory Practice
IU : International Unit
mA : miliabsorbance
mL : mililiter
NCCLS : National Committee for Clinical Laboratory Standards
QC : Quality Control

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SYMBOLS

IVD	Only for invitro diagnostic use
LOT	Lot of manufacturing
R1	Reagent 1
R2	Reagent 2
CONC	Concentration
INGRED	Reagent Ingredients
REF	Reference Number (Catalog No)
SN	Serial Number
	Expiration date
	Storage temperature interval
	Read the directions
	Biological risk

CE

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