

**KIT SPECIFICATIONS:**

Cat. No.	Quantity	Reagent	Storage
AD061	8 x 40 ml	DBIL 1	2-8°C
	4 x 20 ml	DBIL 2	

**INTENDED USE:**

*In Vitro* Diagnostic reagent pack for the quantitative determination of direct Bilirubin in serum and plasma on Hitachi® automated analysers.

**SUMMARY AND EXPLANATION: 1**

Direct bilirubin consists of 2 isomers, mono and di glucuronide. Conjugation with glucuronic acid takes place inside the hepatocytes. The conjugated bilirubin is subsequently excreted in the bile. Direct bilirubin is measured in the investigations of pre-hepatic, post-hepatic and hepatic jaundice. Increased levels are evident with hepatocellular diseases such as hepatitis and cholestasis or decreased drainage such as carcinoma, stricture or stones effecting extra hepatic obstruction.

**PRINCIPLE OF THE TEST: 2,3**

This assay involves measurement of a serum blank in an acid diluent. The subsequent addition of DPD produces a coloured diazonium salt, which is measured at 546 nm. The concentration of diazonium salt formed is proportional to the concentration of direct bilirubin in the sample.

Bilirubin + diazonium ion  $\longrightarrow$  azobilirubin

**WARNINGS AND PRECAUTIONS:**

*For In Vitro Diagnostics Use Only - For Professional Use Only*  
 Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

**Components Colour and Appearance:**

Reagent 1: Clear colourless liquid.

Reagent 2: Pale beige liquid

Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

**Safety precautions:**

This product is not hazardous under EU specifications. Material Safety Data Sheet is available upon request.

**Handling precautions:**

- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.
- Reagent 2 is light sensitive and it must be stored in the dark**

**INSTRUMENTS:**

This assay is designed to run on Hitachi® clinical chemistry analysers. Refer to relevant user's manual or Laboratory internal practice for routine maintenance procedures. *All information is encoded in the barcode. If analyser fails to read or if the barcode is damaged, enter the series of numbers beneath the barcode.*

**COMPONENT COMPOSITION:**

Component	Ingredients	Concentration in Tests
Reagent 1	EDTA - Na2	0.07 mmol/l
	NaCl	6.6 g/l
	Sulfamic acid	70 mmol/l
Reagent 2	2,4-dichlorophenyl diazonium salt (DPD)	0.09 mmol/l
	HCl	130 mmol/l
	EDTA - Na2	0.02 mmol/l

**REAGENT PREPARATION AND STABILITY:**

**Reagent 1 and 2** are ready for use.  
 Before use, mix reagent by gently inverting each bottle.

If stored and handled properly, unopened component is stable until expiry date stated on the label.

*Stability On Board the Instrument: 28 days.*

**Reagent 2 is light sensitive and must be stored in the dark**

**TYPE OF SPECIMEN: 1**

Use serum, free of haemolysis, or EDTA / heparinised plasma as specimen.  
 A morning specimen from a fasting patient should be preferred.

It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Plasma/serum should be separated from cells within 2 hours after collection.

**Stability 1: It is essential to store specimens in the dark, at 2-8°C.** Only in those conditions, stability is maintained for 3 Days.

**TEST PROCEDURE:**

Materials required but not supplied:

Description	Catalog. No.	Description	Catalog. No.
General Chemistry Calibrator	AD983	Hitachi® Analyser	N/A
General Chemistry Control Level 1	AD922	Hitachi® Consumables	N/A
General Chemistry Control Level 2	AD932	General Laboratory Equipment	N/A

**Assay procedure:**

Refer to relevant user's manual for instructions on instrument start-up, loading components and samples, calibration, sample testing procedures, calculating and reporting results.

**Calibration:**

Using recommended Calibrator, calibrate the assay:

- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part.
- When Quality Controls are out of range.

**Quality Control:**

All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.

Controls should be assayed:

- Prior reporting patient results.
- Following any maintenance procedure.
- At intervals established by the QC laboratory programme.

**CALCULATION:**

The analyser automatically calculates the Direct Bilirubin concentration in the sample.  
 (Conversion Factor: Qty in  $\mu\text{mol/l}$  = 17.1 x Qty in mg/dl.)

**EXPECTED VALUES: 1**

Serum	0 - 5.13 $\mu\text{mol/l}$	0 - 0.3 mg/dl
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Each laboratory should establish its own reference range. Direct Bilirubin results should always be reviewed with the patient's medical examination and history.

**PERFORMANCE CHARACTERISTICS:**

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

**Linearity:**

This assay is linear up to 171  $\mu\text{mol/l}$  (10 mg/dl).

For samples with a higher concentration:

- Reassay using, when available, "Rerun" function. Refer relevant user's manual for instructions.
- Or, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result obtained by 2.

**Interfering substances:**

Results of study are as follows:

Haemolysis: Less than 10% interference up to 2.5 g/l Haemoglobin.  
 Lipemia: Less than 10% interference up to 5 g/l Intralipid.

**Sensitivity:**

The Lowest Detectable Level was estimated at 1.1  $\mu\text{mol/l}$ .

**Precision:**

Intra-assay N = 20	Mean (mg/dl)	SD	% CV	Inter-assay N = 20	Mean (mg/dl)	SD	% CV
Level 1	0.76	0.01	1.46	Level 1	0.75	0.01	1.00
Level 2	2.07	0.03	1.30	Level 2	2.13	0.02	0.71

**Method Comparison:**

Using 50 samples, a comparison, between this Direct Bilirubin DPD test (y) and another commercially available test (x), gave the following results:

















$y = 0.95(x) + 0.04 \text{ mg/dl}$	$r = 0.995$	Sample range: 0 to 248 $\mu\text{mol/l}$
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**BIBLIOGRAPHY:**

- Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5<sup>th</sup> ed. 30-54, 601-606 and 966.
- Weigh E, Bach H, Kreig US. Med. Klin. 1975. 70, 664.
- Thomas L. Labor and Diagnose. 4<sup>th</sup> ed. Marburg: Die Med. Verlagsgesellschaft. 1992:235

**SYMBOLS:**

The following symbols are used in the labelling of Audit Diagnostics systems:

	In Vitro Diagnostics		Catalogue No
	Batch Code		Content
	Reagent		Antibody
	Calibrator		Substrate
	Buffer		CE Mark - Device complies with the Directives 98/79/EC
	Storage temperature		Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

**Manufactured By:** AUDIT DIAGNOSTICS, Business & Technology Park, Carrigtwohill, Co. Cork (Ireland)  
 Tel: 00353 - (0) 21 - 4533 652 Fax: 00353 - (0) 21 - 4533 653  
 E-mail: info@auditdiagnostics.ie Website: www.auditdiagnostics.ie



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# BILIRUBIN DIRECT Hitachi® Instruments Settings

## Catalogue No(s): AD061



HITACHI 911/912/917/MDP®

All information is encoded in the barcode. If analyser fails to read or if the barcode is damaged, enter the series of numbers beneath the barcode.

HITACHI 704® (Temperature: 37°C)

CHEMISTRY PARAMETERS	
TEST CODE	[BIL-D]
ASSAY CODE	[2(2POINT)] - [15] - [32]
SAMPLE VOLUME	[30]
R1 VOLUME	[240] - [ ] - [NO]
R2 VOLUME	[60] - [ ] - [NO]
WAVELENGTH	[700] - [546]
CALIB. METHOD	[LINEAR]
STD (1) CONC. POS.	[ ] - [ ]
STD (2) CONC. POS.	[ ] - [ ]
STD (3) CONC. POS.	[0] - [0]
STD (4) CONC. POS.	[0] - [0]
STD (5) CONC. POS.	[0] - [0]
STD (6) CONC. POS.	[0] - [0]
UNITS	[ ]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[500]
SENSITIVITY LIMIT	[0]
ABS LIMIT (INC/DEC)	[0] - [0]
PROZONE LIMIT	[32000] - [1]
EXPECTED VALUE	[ ] - [ ]
INSTRUMENT FACTOR	[1.00]

[ ] User Defined.

HITACHI 717® (Temperature: 37°C)

CHEMISTRY PARAMETERS	
TEST CODE	[BIL-D]
ASSAY CODE	[2(2POINT)] - [24] - [50]
SAMPLE VOLUME	[20] - [ ]
R1 VOLUME	[240] - [ ] - [NO]
R2 VOLUME	[60] - [ ] - [NO]
WAVELENGTH	[700] - [546]
CALIB. METHOD	[LINEAR]
STD (1) CONC. POS.	[ ] - [ ]
STD (2) CONC. POS.	[ ] - [ ]
STD (3) CONC. POS.	[0] - [0]
STD (4) CONC. POS.	[0] - [0]
STD (5) CONC. POS.	[0] - [0]
STD (6) CONC. POS.	[0] - [0]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[500]
SENSITIVITY LIMIT	[0]
ABS LIMIT (INC/DEC)	[0] - [Inc]
PROZONE LIMIT	[0] - [Lower]
EXPECTED VALUE	[ ] - [ ]
PANIC VALUES	[ ] - [ ]
INSTRUMENT FACTOR	[1.00]

[ ] User Defined.

HITACHI 911/912® (Temperature: 37°C)

CHEMISTRY PARAMETERS	
TEST CODE	[BIL-D]
ASSAY CODE	[2 POINT END] - [10]
WAVELENGTH (SUB-MAIN)	[700] - [546]
ASSAY POINT	[15] - [31]
DILUTION	[3] - [14]
SAMPLE VOLUME (µL)	[30] - [ ]
ABS LIMIT	[0] - [Lower]
PROZONE LIMIT	[0] - [UPPER]
REAGENT (µL) R1	[240] - [0] - [0]
R2	[0] - [0] - [0]
R3	[60] - [0] - [0]
R4	[0] - [0] - [0]
CALIBRATION TYPE	[LINEAR] - [2] - [2]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[500]
SENSITIVITY LIMIT	[240]
SI ABS. LIMIT	[-32000] - [32000]
UNIT	[ ] - [ ]
INSTRUMENT A	[1.00]
FACTOR (Y=AX+B) B	[0] - [0]
STD 1	[ ] - [ ]
STD 2	[ ] - [ ]
STD 3	[0] - [0]
STD 4	[0] - [0]
STD 5	[0] - [0]
STD 6	[0] - [0]

[ ] User Defined.

HITACHI 902® (Temperature: 37°C)

CHEMISTRY PARAMETERS	
TEST NAME	[DBIL]
ASSAY CODE	[2POINT END] - [10] - [ ]
ASSAY POINTS	[17] - [35] - [0] - [0]
WAVELENGTH (SUB-MAIN)	[700] - [546]
SAMPLE VOLUME (µL)	[30]
REAGENT (VOL-POS-BOTTLE SIZE) R1	[240] - [ ] - [L]
R2	[0] - [ ] - [S]
R3	[60] - [ ] - [S]
CALIBRATION	[LINEAR] - [0] - [0.00]
CALIB 1 (CONC/POS)	[ ] - [ ]
CALIB 2 (CONC/POS)	[ ] - [ ]
CALIB 3 (CONC/POS)	[0] - [0]
CALIB 4 (CONC/POS)	[0] - [0]
CALIB 5 (CONC/POS)	[0] - [0]
CALIB 6 (CONC/POS)	[0] - [0]
S1 ABS	[0]
K FACTOR	[10000]
K 2 FACTOR	[10000]
K 3 FACTOR	[10000]
K 4 FACTOR	[10000]
K 5 FACTOR	[10000]
A FACTOR	[0]
B FACTOR	[0]
C FACTOR	[0]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[500]
SENSITIVITY LIMIT	[240]
S1 ABS. LIMIT (LOW/HIGH)	[-32000] - [32000]
ABS LIMIT	[0] - [INCREASE]
PROZONE LIMIT	[0] - [Lower]
PROZONE (END POINT)	[35]
EXPECTED VALUES	[ ] - [ ]
INST FACTOR (A - B)	[1] - [0]
KEY SETTING	.....

[ ] User Defined.