

Liquid Reagents – ready to use

# BILIRUBIN AUTO DIRECT

DCA with ATCS\*  
 2 Reagents

Diagnostic reagent for quantitative in vitro determination of direct bilirubin in human serum or plasma on photometric systems.

Ref.No.	Kit Size	Content
D96542B	1 x 12.5 L	1 x 10 L R1 + 1 x 2.5 L R2
D03104B	1 x 1.25 mL	1 x 1 L R1 + 1 x 250 mL R2
D96543	5 x 100 mL	4 x 100 mL R1 + 1 x 100 mL R2
D96544	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
D00547	5 x 25 mL	4 x 25 mL R1 + 1 x 25 mL R2
D00548	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
D58911	10 x 50 mL	10 x 40 mL R1 + 4 x 25 mL R2
D0408917	5 x 62.5 mL	4 x 62.5 mL R1 + 1 x 62.5 mL R2
DA0809	5 x 50 mL	5 x 40 mL R1 + 5 x 10 mL R2
DT1009	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2
DK0708	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DB0909	2 x 150 mL	2 x 120 mL R1 + 2 x 30 mL R2

Additionally offered:

D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P

\* Advanced Turbidity Clearing System; minimizes turbidity caused by lipemia

## TEST PARAMETERS

Method:	Colorimetric, endpoint, increasing reaction, DCA
Wavelength:	546 nm (540 – 560 nm)
Temperature:	20 – 25 °C or 37 °C
Sample:	Serum, heparin plasma
Linearity:	up to 10 mg/dL
Sensitivity:	The lower limit of detection is 0.1 mg/dL

## SUMMARY [1,2]

Bilirubin is a breakdown product of haemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in the blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucuronic acid and the resulting water soluble bilirubin glucuronides are excreted via the bile ducts.

Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60 – 70 % of neonates due to an increased postpartal breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation.

## TEST PRINCIPLE

Direct Bilirubin reacts with diazotized 2,4-dichloroaniline (DCA) to form a red colored azocompound in acidic solution. Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Therefore, the value of unconjugated bilirubin may be estimated from the difference between total bilirubin and direct bilirubin.

## REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
<b>Reagent 1</b>	
EDTA-Na <sub>2</sub>	0.1 mmol/L
NaCl	150 mmol/L
Sulfamic acid	100 mmol/L
<b>Reagent 2</b>	
2,4-Dichlorophenyl-diazonium salt	0.5 mmol/L
HCl	900 mmol/L
EDTA-Na <sub>2</sub>	0.13 mmol/L

## REAGENT PREPARATION

### Substrate Start:

The reagents are ready to use.

### Sample Start:

Not possible.

## REAGENT STABILITY AND STORAGE

Conditions: Avoid contamination.  
 Close immediately after use.  
 Reagent 2 must be protected from light!  
 Do not freeze the reagents.

Storage: at 2 – 8 °C  
 Stability: up to the expiration date

## SAMPLE STABILITY AND STORAGE

It is very important to store the sample protected from light!

Stability [3]:	at 20 – 25 °C	1 day
	at 4 – 8 °C	7 days
	at - 20 °C *	6 months

\* in case of immediate freezing. Freeze only once!

Discard contaminated specimens.

## MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)  
 General laboratory equipment

## MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

### Substrate Start:

Pipette into test tubes	Blank	Calibr.	Sample
Reagent 1	1000 µL	1000 µL	1000 µL
Sample	-	-	50 µL
Calibrator	-	50 µL	-
Dist. water	50 µL	-	-
Mix. Incubate for 3 – 5 min. (20 – 25 °C / 37 °C) and read absorbance A1 against reagent blank. Then add:			
Reagent 2	250 µl	250 µl	250 µl
Mix. Incubate for 5 min. (37°C) or 10 min. (20 – 25 °C) and read absorbance A2 against reagent blank. Calculate: $\Delta A = A2 - A1$ .			

## CALCULATION

$$\text{Bilirubin [mg/dL]} = \frac{\Delta A \text{ sample}}{\Delta A \text{ calibrator}} \times \text{conc. cal. [mg/dL]}$$

## UNIT CONVERSION

$$\text{Bilirubin [mg/dL]} \times 17.1 = \text{Bilirubin [\mu mol/L]}$$

## REFERENCE RANGE [1] \*

Adults and children ≤ 0.2 mg/dL (≤ 3.4 µmol/L)

\* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

## PERFORMANCE CHARACTERISTICS

### LINEARITY, MEASURING RANGE

The test has been developed to determine bilirubin concentrations within a measuring range from 0.1 – 10 mg/dL. When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the results multiplied by 2.

### SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 0.1 mg/dL

### PRECISION (at 37°C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.36	0.01	3.12
Sample 2	0.76	0.01	1.46
Sample 3	2.07	0.03	1.30

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.35	0.01	3.34
Sample 2	0.75	0.01	1.00
Sample 3	2.13	0.02	0.71

### SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Hemoglobin	50 mg/dL
Triglycerides	1000 mg/dL
Naproxen	1 mmol/L

For further information on interfering substances refer to Young DS [5].

### METHOD COMPARISON

A comparison between Dialab Bilirubin Auto Direct (y) and a commercially available test (x) using 85 samples gave following results:  $y = 0.95x + 0.04$  mg/dL;  $r = 0.995$ .

### CALIBRATION

The assay requires the use of a Bilirubin Standard or Calibrator. We recommend the Dialab multi calibration serum **Diacal Auto**. This method has been standardized against the manual Jendrassik-Gróf test.

### QUALITY CONTROL

All control sera with bilirubin values determined by this method can be used.

We recommend the Dialab controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).

Each laboratory should establish corrective action in case of deviations in control recovery.

### AUTOMATION

Special adaptations for automated analyzers can be made on request.

### WARNINGS AND PRECAUTIONS

- Reagent 1 and 2: Warning.  
 H290: May be corrosive to metals.  
 P234: Keep only in original container.  
 P390: Absorb spillage to prevent material damage.
- In very rare cases, samples of patients with gammopathy might give falsified results [6].
- Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
- For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
- For professional use only!

### WASTE MANAGEMENT

Please refer to local legal requirements.

### REFERENCES

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- Bakker AJ, Mücke M. Gammopathy interfere in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

