

## Bilirubin Direct



### Order Information

Cat. No.  
OMR1041

Kit Configuration  
Reagent 1: 2 x 40 mL  
Reagent 2: 1 x 10 mL

### Summary

Bilirubin is a breakdown product of hemoglobin. 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. Common bilirubin methods detect either total bilirubin or direct bilirubin. Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Unconjugated bilirubin can therefore be estimated as the difference between total bilirubin and direct bilirubin.

### Method

Modified method

#### Principle

Sulfanilic acid reacts with sodium nitrite to produce diazotized sulfanilic acid (dialzo). Direct bilirubin couples with dialzo to produce azobilirubin. The intensity of the color produced is directly proportional to the amount of direct bilirubin present in the sample.

### Reagents

Storage Instructions and Reagent Stability

Reagent up to the end of the indicated month of expiry, if stored at 2° - 30°C, protected from light and contamination is avoided. Do not freeze the reagents!

Reagent 1: Sulphanilic

Solution Reagent 2: Nitrite

Solution

#### Composition

Sulphanilic acid 7 gm/L, Conc. HCL 270 mL/L, Sodium Nitrite 7.0 g/L.

### Warning and Precautions

1. Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
2. Take off immediately all contaminated clothing.
3. Wear suitable gloves and eye/face protection.
4. Always use safety pipettes to pull the reagents into a pipette.
5. Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.
6. For professional use only!

### Waste Management

Please refer to local legal requirements.

### Reagent Preparation

Reagents are ready to use.

### Materials required but not provided

NaCl solution 9 g/L

General laboratory equipment

### Specimen

Serum, heparin, plasma or EDTA plasma separate at the latest 1h after blood collection from cellular contents.

#### Stability in

plasma/Serum 4 days

at 2 -8°C

30 days at -20°C

### Assay Procedure

Wavelength 546 nm

Optical path 1 cm

Temperature 37°C

Measurement Against sample blank

### Direct Bilirubin

	Sample Blank	Test
Reagent 1	1000 µL	1000 µL
Reagent 2	---	50 µL
Sample	50 µL	50 µL
Mix, Incubate for exactly 60 seconds at 37°C. Read absorbance against sample blank.		

### Calculation

Note: Take  $\Delta A$  (sample) and multiply by the corresponding factor from below:

$$\Delta A (\text{sample}) = \text{Sample Test } (A_2) - \text{Sample Blank}$$

$$(A_1) \text{ Bilirubin (Direct) mg/dL} = \Delta A (\text{sample}) \times$$

factor (21)

### Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples. Each laboratory should establish corrective action in case of deviations in control recovery.

### Performance Characteristics and Measuring Range

The test has been developed to determine bilirubin within a measuring range from 0.5-30 mg/dL. When values exceed this range samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

### Specificity/Interferences

No interference was observed by triglycerides up to 800 mg/dL.

### Sensitivity/Limit of Detection

The lower limit of detection is 0.1 mg/dL.

### Linearity

The higher limit of detection is 30 mg/dL.

## Precision

### Direct Bilirubin

Intra-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.73	0.07	9.78
Sample 2	1.89	0.05	2.39

Inter-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample1	0.76	0.04	5.34
Sample 2	1.70	0.07	3.93

## Method Comparison

A comparison of Nucleus Diagnosys Bilirubin (y) with a commercially available test (x) using 15 samples gave following results:

$$y = 1.041x - 0.027; r^2 = 0.975$$

## Reference Range

Direct Bilirubin	mg/dL
Adults and children	<0.2

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

## Literature

1. Thomas L ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998: p. 192 - 202.
2. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.
4. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962;6:570-8.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.

MANUFACTURED BY: NUCLEUS DIAGNOSYS LLP, INDIA

Marketed By: ORCHARD MEDICAL,

DIAMOND ARCADE, 68 JESSORE ROAD,

1<sup>st</sup> FLOOR, UNIT No. 110 & 112, KOLKATA - 700 055

CUSTOMER CARE No: 84200 69980

CUSTOMER CARE E-MAIL: sales.orchardmedical@gmail.com