

# Bilirubin (Total & Direct) Kit

## CliniQuant - FSR



Diagnostics

DCA Method

For *in vitro* diagnostic use  
Read this pack insert thoroughly before use

REF	Pack Size	R1 Bilirubin Total Reagent	R2 Bilirubin Direct Reagent	R3 Sodium Nitrite Reagent
BILFSR-01	4 x 50ml	2 x 50ml	2 x 50ml	1 x 6ml
BILFSR-02	2 x 200ml	1 x 200ml	1 x 200ml	1 x 12ml
BILFSR-03	4 x 250ml	2 x 250ml	2 x 250ml	2 x 25ml
BILFSR-04	2 x 500ml	1 x 500ml	1 x 500ml	2 x 25ml

### INTENDED USE

This reagent is intended for quantitative determination of total and direct bilirubin in human serum or plasma.

### CLINICAL SIGNIFICANCE

Bilirubin a breakdown product of hemoglobin in the reticulo-endothelial system is transported to the liver in association with albumin. This bilirubin is water insoluble known as indirect or unconjugated bilirubin. In liver, bilirubin is conjugated to glucuronic acid to form direct bilirubin or conjugated bilirubin and excreted into intestine via biliary system.

TOTAL BILIRUBIN = INDIRECT BILIRUBIN + DIRECT BILIRUBIN

Total Bilirubin is elevated in obstructive conditions of bile duct, hepatitis, cirrhosis of liver and hemolytic disorders. Indirect Bilirubin is elevated by pre-hepatic causes such as hemolytic disorders or liver diseases. Monitoring of indirect bilirubin in neonates is of special importance as it is the indirect (free) bilirubin bound to albumin that is able to cross the blood brain barrier more easily increasing the danger of cerebral damage.

### PRINCIPLE OF THE METHOD

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to produce pink colored azobilirubin that is proportional to the concentration of bilirubin. Direct bilirubin being water soluble reacts directly in acidic media, however indirect bilirubin is solubilised using surfactant and then reacts similar to direct bilirubin.

### KIT COMPONENTS

R1 - Bilirubin Total Reagent : Surfactant 1%, Sulfanilic Acid 0.8 g/l, HCl 10 ml/l

R2 - Bilirubin Direct Reagent : Sulfanilic acid 0.8 g/l

R3 - Sodium Nitrite Reagent : Sodium Nitrite 28.98mmol/l

### MATERIALS REQUIRED BUT NOT PROVIDED

Laboratory instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi automated, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/ rack, heating bath, controls, saline.

### REAGENT PREPARATION, STORAGE & STABILITY

Stability: unopened bottle up to expiration date on labels +15 to +30°C.

Keep away from direct light sources.

### REAGENT DETERIORATION

Discard the working reagent if it fails to achieve assigned assay values of fresh control sera.

### WARNINGS AND PRECAUTIONS

1. Reagent may contain some non-reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
2. Specimens should be considered infectious and handled appropriately.
3. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

### SPECIMEN

Serum, plasma (heparinate only). Bilirubin in serum is stable for one month at -20°C or 7 days when stored in dark at 2-8 °C.

### Programme Parameter for MERILYZER CliniQuant

Bilirubin	Total Direct	
	End Point	End Point
Factor	12	10
Filter - 1 (nm)	546	546
Filter - 2 (nm)	670	670
Temperature	37 °C	37 °C
Volume (µl)	500	500
Delay Time (Sec)	5	5
Reaction Direction	Increase	Increase
Reference Low	0.1	0
Reference High	1.2	0.3
Linearity Limit	30	20



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### TEST PROCEDURE – Total Bilirubin

Dispense	Blank	Test
Reagent 1	1 ml	1 ml
Reagent 3	25 µl	25 µl
Distilled water	100 µl	-
Sample	-	100 µl

Mix, incubate for 5 min at 37°C. Read absorbance at 546/670 nm against reagent blank.

### TEST PROCEDURE – Direct Bilirubin

Dispense	Blank	Test
Reagent 2	1 ml	1 ml
Reagent 3	25 µl	25 µl
Distilled water	100 µl	-
Sample	-	100 µl

Mix, incubate for 1 min at 37°C. Read absorbance at 546/670 nm against reagent blank.

### RESULT CALCULATION

Serum/plasma:

Total Bilirubin mg/dl = Abs. of Test- Abs. of Blank x 12

Direct Bilirubin mg/dl = Abs. of Test- Abs. of Blank x 10

SI conversion factor: 1 mg/dl x 17.1 = 1 µmol/l

### EXPECTED VALUES

Total Bilirubin:

Adults: 0.1 – 1.2 mg/dl OR 1.7 – 20.5 µmol/l

Infants: 1.2 – 12 mg/dl OR 20.5 – 205 µmol/l

Direct Bilirubin:

Adults & Infants: 0 – 0.3 mg/dl OR 0 – 5.1 µmol/l

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

### QUALITY CONTROL AND CALIBRATION

It is recommended to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

When using the recommended Calibrator (BioCal), calibrate the assay:

- When using a new reagent or lot
- When QC values are out of range

### PERFORMANCE CHARACTERISTICS

#### 1. Linearity

The linearity of Total Bilirubin is up to 30 mg/dl or 513 µmol/l

The linearity of Direct Bilirubin is up to 20 mg/dl or 342 µmol/l

#### 2. Sensitivity/ Limit of detection (LOD)

The limit of detection of Total Bilirubin is 0.2 mg/dl.

The limit of quantification of Total Bilirubin is 0.6 mg/dl.

The limit of detection of Direct Bilirubin is 0.06 mg/dl.

The limit of quantification of Direct Bilirubin is 0.17 mg/dl.

#### 3. Interferences

Gross hemolysis and/ or lipaemia may cause falsely low and/ or elevated results.

#### 4. Precision

Intra-assay precision

	Mean	SD	CV
n = 20	mg/dl	mg/dl	%
sample 1	0.77	0.03	3.68
sample 2	4.04	0.05	1.18

Inter-assay precision

	Mean	SD	CV
n = 20	mg/dl	mg/dl	%
sample 1	0.69	0.03	4.69
sample 2	3.97	0.17	4.4

#### 5. Methods Comparison

Comparison was done between reference Bilirubin Reagent and CliniQuant - FSR Bilirubin Reagent (test)

N = 21  $y = 1.934x - 0.024$

$r^2 = 0.961$

#### LIMITATIONS

After 2 minutes, indirect bilirubin reacts slowly with diazotized dichloroaniline leading to over-estimated values.

#### WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for correct waste disposal

#### REFERENCES

- Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia, W.B. Saunders Company, 1994, p. 1458 - 1470.
- Data on file: Meril Diagnostics.

IFU/BILFSR01/00

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Symbols used on Meril Diagnostics labels:

 REF	Catalogue No.		Attention See Instruction for Use
 LOT	Batch No.		In vitro Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged
	Authorized European Representative in the European Community		