## LIQUIZYME

## **DIRECT BILIRUBIN**

(DMSO Method)

| Code   | Product Name               | Pack Size |
|--------|----------------------------|-----------|
| LS055A | Liquizyme Direct Bilirubin | 240 ml    |
| LS055B | Liquizyme Direct Bilirubin | 1200 ml   |

#### Intended Use

Diagnostic reagent for quantitative *in vitro* determination of Bilirubin in human serum.

## **Clinical Significance**

Bilirubin is a breakdown product of haemoglobin. Bilirubin formed in the reticulo endothelial system is transported bound by albumin to the liver. This bilirubin is water insoluble and is known as indirect or unconjugated bilirubin. In the liver, bilirubin is conjugated to glucuronic acid to form direct bilirubin. Conjugated bilirubin is excreted via the biliary system into the intestine. Here it is metabolised by bacteria to urobilinogen & stercobilinogen.

#### **Principle**

In the determination of Bilirubin Total, Bilirubin is coupled with diazotized sulfanilic acid in the presence of ethylene glycol and dimethylsulfoxide as solvents to produce an intensely colored diazo dye. The intensity of colour of this solution is proportional to the concentration of the bilirubin total in the sample.

#### Reaction

#### **Direct Bilirubin**

Bilirubin + Sulphanilic acid + Sodium Nitrite 

→ Azobilirubin

#### Contents:

## Reagent 1: Direct Bilirubin Reagent

Buffer : < 15 mmol/l Sulphanilic Acid : > 20 mmol/l

# Reagent 2 : Direct Nitrite Reagent Sodium Nitrite : < 10 mmol/l

Reagent 3: Bilirubin Artificial Standard : 10 mg/dl

Ready to use

#### **Unit Conversion**

 $mg/dl \times 16.95 = \mu mol/l$ 

#### Normal Value:

**Serum** Direct Bilirubin : upto 0.3 mg/dl

Each Laboratory should establish it's own normal range representing its patient population.



## **Reagent Preparation**

Reagents are liquid, ready to use.

## Stability and storage

The unopened reagents are stable till the expiry date stated on the bottle and label when stored at room temperature.

#### **Direct Bilirubin**

#### Performance Data

Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification : 0.0052 mg Linearity : 20 mg/dl

Measuring range : 0.0052 – 20 mg/dl

| Intra-assay precision | Mean    | SD      | CV   |
|-----------------------|---------|---------|------|
| Within run (n=20)     | (mg/dl) | (mg/dl) | (%)  |
| Sample 1              | 0.251   | 0.01    | 3.59 |
| Sample 2              | 1.15    | 0.01    | 0.47 |
| Inter-assay precision | Mean    | SD      | CV   |
| Run to run (n=20)     | (mg/dl) | (mg/dl) | (%)  |
| Sample 1              | 1.16    | 0.01    | 1.02 |

## Comparison

A comparison between Beacon Direct Bilirubin(y) and a commercially available test (x) using 20 samples gave following results:

y = 0.993 x + 0.011 mg/dl

r = 0.999

#### Linearity:

This procedure is linear upto 20 mg/dl. If the values exceed this limit, dilute the sample with normal saline (NaCl 0.9%) and repeat the assay. Multiply result by dilution factor.

#### Interferences

Following substances do not interfere:

haemoglobin up to 7.5 g/l, triglycerides up to 1500 mg/dl.

#### **Warning And Precautions**

For in vitro diagnostic use. To be handled by entitled and professionally educated person.

#### Procedure:

Pipette into clean dry test tubes labeled as Blank (B), and Test (T):

## **Direct Bilirubin**

| Addition Sequence          | (B)     | (T)     |
|----------------------------|---------|---------|
| Direct Bilirubin Reagent   | 1000 μΙ | 1000 μΙ |
| Direct Bilirubin Activator | -       | 20 μΙ   |
| Serum                      | 50 μl   | 50 μΙ   |

Mix well and incubate at 37°C for exactly 5 minutes. Measure the absorbance of the Test Samples (Abs. T) immediately against their respective Blanks.

#### Calculation

## With factor:

Direct Bilirubin = OD of test - OD of sample blank x Factor (20)

## With artificial standard:

Bilirubin Concentration (mg/dl) =  $\frac{\text{OD test - OD of sample Blank}}{\text{OD of standard}} \times 10$ 

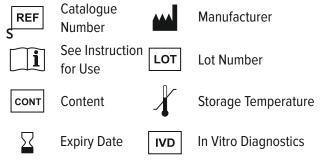
## **Assay Parameters For Photometers**

| Mode                            | End Point |
|---------------------------------|-----------|
| Wavelength                      | 546       |
| Sample Volume (μΙ)              | 50        |
| Direct Bilirubin Reagent (μΙ)   | 1000      |
| Direct Bilirubin Activator (μl) | 20        |
| Incubation Time                 | 5 min     |
| Reaction temperature (°C)       | 37        |
| Linearity Low (mg/dl)           | 0.0052    |
| Linearity High (mg/dl)          | 20        |
| Blank with                      | Serum     |
| Standard Concentration          | 10 mg/dl  |
| Unit                            | mg/dl     |

## References

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## ymbols Used On Labels



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