GENESIS

# Instructions For Use

## Bilirubin (Jendrassik & Groff (T&D))

| Cat no. | size |
|---|---|
| 1102 101 | 265 ml |

**INTENDED USE**

Bilirubin Reagent in intended for in vitro Quantitative diagnostic determination of total and direct bilirubin.

**DIAGNOSTIC CHARACTERISTICS**

Bilirubin is a waste product derived from the heme moiety of the hemoglobin released from senescent or damaged erythrocytes, that are destroyed in the reticuloendothelial cells. After production, bilirubin is transported to the liver in association with albumin. Inside the hepatocytes bilirubin is conjugated with glucuronic acid and it is excreted into bile.

A number of inherited and acquired diseases affect production, uptake, metabolism, and excretion of bilirubin, resulting in hyperbilirubinemia.

Unconjugated hyperbilirubinemia is seen in newborns (physiological jaundice), in increased red cell destruction (hemolytic anemia, extensive hematoma), in ineffective erythropoiesis and in some rare genetic diseases (Gilbert's syndrome, Crigler-Najjar syndrome).

Conjugated hyperbilirubinemia is associated to a decreased excretion of bile due to liver diseases (hepatitis or cirrhosis) or to intrahepatic or extra hepatic cholestasis.

Jaundice is a clinical manifestation of hyperbilirubinemia, consisting of deposition of bile pigments in the skin, resulting in a yellowish staining of the skin and mucous membranes.

**PRINCIPLE OF THE METHOD**

In the presence of caffeine, the total bilirubin concentration is determined by the reaction with diazotized sulphanilic acid to produce an intensely colored diazo dye (560-600 nm). The intensity of color of this dye formed is proportional to the concentration of total bilirubin. Direct bilirubin is determined in absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red colored azobilirubin; the color intenisity of which measured at 546 nm is proportional to the concentration of the direct bilirubin in the sample.

Sulfanilic acid + $NaNO_2$ $\xrightarrow{HCL}$ Diazotized sulfanilicacid

Bilirubin + Diazotized sulfanilic acid $\xrightarrow{pH\ 1.4}$ Azobilirubin

**COMPOSITION**

| | | |
|---|---|---|
| **Sulphanilic Reagent ($R_1$)** | Sulfanilic acid | 5 mmol/L |
| | HCL | 0.3 N |
| **SOD Nitrire Reagent ($R_2$)** | Sodium nitrite | 3 mmol/L |
| **Caffiene Reagent ($R_3$)** | Caffiene | 45 mmol/L |
| | Sodium benzoate | 20 mmol/L |
| **Alkaline Reagent ($R_4$)** | Sodium tartarate | 25 mmol/L |
| | Sodium hydroxide | 1.5 N |

**STORAGE**

Store at 20-25ºC.

Reagents are stable until the expiry date shown on the vial label .

**REAGENT PREPARATION**

Reagents provided are ready to use.

**ADDITIONAL EQUIPMENT**

Analyzer, spectrophotometer or photometer able to read at 578nm and 546nm.

**SPECIMEN**

1.Serum collected by standard procedures.

2.Bilirubin in serum is stable for 2 days at 2-8ºC if serum is protected from light.

3.Discard contaminated specimens.

**Precautions and Warnings**

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

**Symbols in Product Labeling**

| | | | |
|---|---|---|---|
| EC REP | Authorized Representative | | Expiration date |
| IVD | For in-vitro diagnostic use | | CAUTION, consult instructions for use |
| REF | Catalogue number | | |
| LOT | Lot number | | Manufactured by |

**PROCEDURE:**

| **Total Bilirubin** | | |
|---|---|---|
| | Serum Blank | Sample |
| **Sulphanilic Reagent (R1)** | 200 µL | 200µL |
| **Sod Nitrire Reagent (R2)** | - | 1 drop |
| **Caffiene Reagent (R3)** | 1.0 mL | 1.0 mL |
| **Sample** | 200 µL | 200 µL |
| Mix thoroughly and incubate the tubes for **10 minutes** at room temperature (16-25ºC).then add | | |
| **Alkaline Reagent (R4)** | 1.0 ml | 1.0 ml |
| Mix and incubate for **5 minutes** at 20 – 25ºC. Measure the absorbance (A) of the Sample at 578 nm against the sample blank. The color is stable for at least **30 minutes**. | | |

| **Direct Biliubin** | | |
|---|---|---|
| | Serum Blank | Sample |
| **Sulphanilic Reagent (R1)** | 200 µL | 200µL |
| **Sod Nitrire Reagent (R2)** | - | 1 drop |
| **Saline 0.9% NaCl** | 2.0 ml | 2.0 ml |
| **Sample** | 200 µL | 200 µL |
| Mix and incubate for exactly **5 minutes** at 20 – 25ºC. Measure absorbance of sample (A sample) against sample blank at 546 nm. | | |

**CALCULATIONS**

The Bilirubin concentration in the sample is calculated using the following general formula:

Total bilirubin (mg/dl) = (A sample –A sample blank) x 10.8

Direct bilirubin (mg/dl) = ( A sample–A sample blank) x 14.4

**REFERENCE VALUES**

| | |
|---|---|
| Total Bilirubin | Up to 1.0 mg/dL =17.0µmol/L |
| Direct Bilirubin | Up to 0.2 mg/dL = 3.4 µmol/L |

**QUALITY CONTROL**

It is recommended to use the Control Serum level I and II to verify the performance of the measurement procedure.

**METROLOGICAL CHARACTERISTICS**

−Detection limit: (Total Bilirubin): 0.03 mg/dL

(Direct Bilirubin): 0.02 mg/dL

− Linearity limit: 20.0 mg/dL.

− Repeatability (within run):

Total Bilirubin / Direct Bilirubin

| Mean Concentration | cv | n | Mean Concentration | cv | n |
|---|---|---|---|---|---|
| 0.59 mg/dL | 3 % | 20 | 0.77 mg/dL | 1.2 % | 20 |
| 6.74 mg/dL | 1% | 20 | 1.36 mg/dL | 0.5% | 20 |

− Reproducibility (run to run):

Total Bilirubin / Direct Bilirubin

| Mean Concentration | cv | n | Mean Concentration | cv | n |
|---|---|---|---|---|---|
| 0.59 mg/dL | 3.6 % | 20 | 0.77 mg/dL | 2.3 % | 20 |
| 6.74 mg/dL | 3.3% | 20 | 1.36 mg/dL | 0.9% | 20 |

**INTERFERENCES**

Hemoglobin (10 g/L) does not interfere.

Lipemia (triglycerides > 15 g/L) may intefere.

Other drugs and substances may interfere

**BIBLIOGRAPHY**

1. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.

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