Bilirubin Auto Total FS*

Order Information

Cat. No.	Kit size				
1 0811 99 10 021	R1	5 x 20 mL	+	R2	1 x 25 mL
1 0811 99 10 026	R1	5 x 80 mL	+	R2	1 x 100 mL
1 0811 99 10 023	R1	1 x 800 mL	+	R2	1 x 200 mL
1 0811 99 10 704	R1	8 x 50 mL	+	R2	8 x 12.5 mL
1 0811 99 10 917	R1	8 x 60 mL	+	R2	8 x 15 mL
1 0811 99 10 930	R1	4 x 20 mL	+	R2	2 x 10 mL
1 0811 99 90 314	R1	10 x 20 mL	+	R2	2 x 30 mL

Intended Use

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

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 glucoronic acid and the resulting water soluble bilirubin glucoronic acid is 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 postpartum 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. Therefore, the value of unconjugated bilirubin may be estimated from the difference between total bilirubin and direct bilirubin. [1,2]

Method

Photometric test using 2,4-dichloroaniline (DCA)

Direct bilirubin in presence of diazotized 2,4-dichloroaniline forms a red colored azocompound in acidic solution. A specific mixture of detergents enables a safe determination of the total bilirubin [3].

Reagents

Components and Concentrations

R1:	Phosphate buffer	50 mmol/L
	NaCl	150 mmol/L
R2:	2,4-Dichloroaniline	5 mmol/L
	HCl	130 mmol/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze reagents and protect them from light.

Warnings and Precautions

1. ⚠ Reagent 1: Warning. H290 May be corrosive to metals. H315 Causes skin irritation. H319 Causes serious eye irritation. H410 Very toxic to aquatic life with long lasting effects. P234 Keep only in original packaging. P264 Wash hands and face thoroughly after handling. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/eye protection. P337+P313 If eye irritation persists: Get medical advice/attention. P391 Collect spillage.
2. ⚠ Reagent 2: Warning: H290 May be corrosive to metals. H319 Causes serious eye irritation. P234 Keep only in original packaging. P264 Wash hands and face thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice/attention. P390 Absorb spillage to prevent material damage.
3. In very rare cases, samples of patients with gammopathy might give falsified results [4].
4. Eltrombopag medication leads to falsely low or high results in patient samples.
5. 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.
6. For professional use only.

Waste Management

Refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum or heparin plasma

Protect sample from light.

Stability [5]:

1 day	at	20 – 25°C
7 days	at	4 – 8°C
6 months	at	–20°C

in case of immediate freezing.

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	545/658 nm
Temperature	37°C
Measurement	Endpoint
Sample/Calibrator	2 µL
Reagent 1	80 µL
Reagent 2	20 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance 1	Cycle 17/18 (231/244 s)
Absorbance 2	Cycle 41/42 (586/600 s)
Calibration	Linear

Calculation

With calibrator

$$\text{Bilirubin [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal}} \times \text{Conc. Cal [mg/dL]}$$

Calibrators and Controls

DiaSys TruCal U is recommended for calibration. TruCal U calibrator values have been made traceable to the NIST SRM 916 reference material. Use DiaSys TruLab N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range up to 30 mg/dL. When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Limit of detection**	0.03 mg/dL

Interfering substance	**Interferences ≤ 10% up to**
Ascorbic acid	30 mg/dL
Hemoglobin	500 mg/dL
Lipemia (triglycerides)	1000 mg/dL
For further information on interfering substances refer to Young DS [6,7].	

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	0.42	3.21	7.22
CV [%]	1.78	1.66	1.09
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	1.37	5.34	7.17
CV [%]	2.25	1.81	1.19

Method comparison (n=100)	
Test x	Competitor Bilirubin Auto Total
Test y	DiaSys Bilirubin Auto Total FS
Slope	1.04
Intercept	0.045 mg/dL
Coefficient of correlation	0.9998

** lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Conversion Factor

Bilirubin [mg/dL] x 17.1 = Bilirubin [µmol/L]

Reference Range [1]

	[mg/dL]	[µmol/L]
Neonates		
24 h	< 8.8	< 150
2nd day	1.3 – 11.3	22 – 193
3rd day	0.7 – 12.7	12 – 217
4th – 6th day	0.1 – 12.6	1.7 – 216
Children		
>1 month	0.2 – 1.0	3.4 – 17
Adults	0.1 – 1.2	1.7 – 21

Each laboratory should check if the reference ranges 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.
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3. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962; 6: 570-8.
4. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.
6. 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.
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* Fluid Stable