Bilirubin Auto Total FS
Bilirubin Auto Total Reagent Test Kit

Intended Use
Diagnostic reagent for in vitro quantitative determination of total bilirubin in human serum or Plasma on photometric analyzers.

Order Information

Item Code	Pack Size
108119934840	R1: 4 x 60 mL, R2 : 4 x 15 mL

Summary [1,2]
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 glucoronides 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) hyper-bilirubinemia 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 conj ugated, water soluble bilirubin . Unconj ugated bilirubin can therefore be estimated as the difference between total bilirubin and direct bilirubin.

Method
Photometric test

Principle
In acidic solution, direct bilirubin forms a red colored azocompound with diazotized 2, 4-dichloroaniline. A specific mixture of detergents enables a safe determination of the total bilirubin.

Reagents

Components and Concentrations

R1:	Hydrochloric Acid	15ml/Lit
	Sulphanilic Acid	7 g/L
	Dimethyl Sulphoxide	200 g/L
R2:	Sodium Nitrite	7 g/L
	Preservatives & Stabilizers	q.s.

Storage Instructions and Reagent Stability
The reagents are stable up to the en d of the indicated month of expiry, if st ored at 2 – 8°C and contamination is avoided. Do not freeze the reagents!
Reagent 2 must be protected from light!

Warnings and Precautions

1. Reagent 1 and 2: May be corrosive to metals. Causes serious eye irritation. Keep only in original container. Wear protective gloves/protective clothing/eye protection/face protection. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. Absorb spillage to prevent material damage.
2. Reagent 2: Wash hands and face thoroughly after handling.
3. In very rare cases, s amples of patients with gammopathy might give falsified results [6].
4. 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.
5. For professional use only!

Waste Management
Please refer to local legal requirements.

Reagent Preparation
The reagents are ready to use.

Traceability
The calibrator values are traceable against IFCC reference material NIST SRM 916.

Materials required but not provided
NaCl solution 9 g/L
General laboratory equipment
TruCal U (591009910064)
TruLab N (590009910061)
TruLab P (590509910061)

Specimen
Serum or heparin plasma
It is very important to store the sample protected from light!

Stability [3]:	1 day	at	20 – 25°C
	7 days	at	4 – 8°C
	6 months	at	– 20°C

If frozen immediately! Freeze only once!
Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	546 nm (540 – 560 nm)
Optical path	1 cm
Temperature	20 – 25°C/37°C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	25 µL
Dist. Water	25 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incub ate for 5 min. a t 37 °C or 10 min. at 20 – 25°C, read absorbance A1, then add:		
Reagent 2	250 µL	250 µL
Mix, incubate for 5 min. at 37 °C, or 10 min . at 20 – 25°C, then read absorbance A2.		

ΔA= [(A2 – A1) sample or calibrator]

Calculation
With calibrator

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

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

Calibrators and Controls
For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The assigned calibrator values for total bilirubin have been made traceable to the NIST SRM 916 reference material. DiaSys TruLab N and P controls should be assayed for internal quality control. Each laboratory should establish correctI've action in case of deviations in control recovery.

Performance Characteristics

Measuring range

The test has been developed to determine bilirubin concentrations within a measuring range from 0.1 – 30 mg/dL. When values exceed this ra nge samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Clinical Interpretation

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, hemoglobin up to 500 mg/ dL , naproxen up to 1 mmol/L and lipemia up to 2000 mg/dL triglycerides when measured using a trigylceride concentrate and up to 1000 mg/dL triglycerides when measured using Intralipid. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 0.07 mg/dL.

Precision (at 37 °C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.89	0.03	3.05
Sample 2	1.02	0.02	2.32
Sample 3	4.83	0.05	0.95

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.87	0.02	2.74
Sample 2	1.15	0.04	3.49
Sample 3	4.65	0.13	2.86

Method Comparison

A comparison of DiaSys Bilirubin Auto Total FS (y) with a commercially available test (x) using 247 samples gave following results: y = 1.003 x - 0.001 mg/dL; r = 1.000

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 populati on and determine own reference ranges if necessary.

Literature

1. Thomas Led. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH -Books V erlagsgesellschaft, 1998. p 192–202.
2. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz Textb ook of Clinical Chemistry. 3 rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
3. Guder WG, Zawta Betal. 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.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChem LabMed 2007;45(9):1240 –1243.

Notes on Symbols and Marks

	Consult instruction for use
	Use-by date
LOT	Batch code
REF	Catalogue number
	Caution
	Manufacturer
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CONT.	The pack contains
	Recycle
	Date of manufacture

ISO 9001, ISO 13485 and ICMED 13485 Certified Company

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