TOTAL BILE ACIDS (TBA)

5TH GENERATION
ENZYMATIC COLORIMETRIC
RX SERIES

INTENDED USE

For the quantitative determination of Total Bile Acids in Animal serum and plasma. This product is suitable for use on RX **series** instruments which includes the RX **daytona** and the RX **imola**.

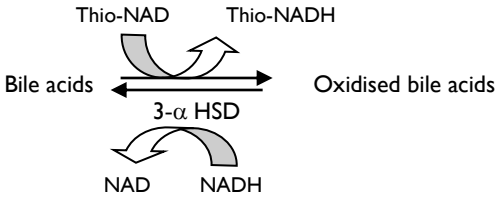
Cat. No.

BI 3863	R1. Reagent 1	2 x 18 ml
	R2. Reagent 2	2 x 8 ml

GTIN: 05055273200720

ASSAY PRINCIPLE [1,2]

In the presence of Thio-NAD, the enzyme 3-I hydroxysteroid dehydrogenase (3-I HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-I HSD can convert 3-keto steroids and Thio-NADH to bile acids and Thio-NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405nm.



SAMPLE COLLECTION AND STORAGE

Serum, EDTA/Lithium heparin plasma. Serum or plasma samples are stable for 1 week at +2 to +8 °C, or for 3 months at –20°C.

REAGENT COMPOSITION

Contents		Initial Concentration of Solutions
R1.	**Reagent 1**	
	Goods buffer, pH 4.0	
	Thio-NAD	1.0g/l
	Sodium Azide	0.05%(w/v)
R2.	**Reagent 2**	
	Goods Buffer, pH 9.3	
	NADH	6.0g/l
	3-α-HSD	12KU/l
	Sodium Azide	0.05% (w/v)
	Stabilisers	

SAFETY PRECAUTIONS AND WARNINGS

Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1 and R2 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium-Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Please dispose of all biological and chemical materials according to local guidelines.

Health and Safety data sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Reagent 1
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R2. Reagent 2
Contents ready for use. Stable up to expiry date when stored at +2 to +8°C.

MATERIALS PROVIDED

Reagent 1
Reagent 2

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multi-sera Level 2, (Cat. No. HN 1530) and Level 3, (Cat. No. HE 1532).
Randox Calibration Serum Level 3, (Cat. No. CAL 2351).

PROCEDURE NOTE

This reagent should not be used if exposed to temperatures above +25°C for greater than 8 hours, as the accuracy of the assay will be affected.

The Chemistry parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Laboratories Technical Services, Northern Ireland
+ 44 (0) 28 9445 1070.

Bile acids and Lipase (Randox Cat. No. LI 3837) should not be tested in the same run on the RX **series** instruments.

Bile acids should not be run immediately after Fructosamine (Randox Cat. No. FR4030) on the RX **daytona** and RX **imola** analysers. Alternatively, a carry-over avoidance procedure can be set up. For details of this procedure, please contact Randox Laboratories Technical Services, Northern Ireland
+ 44 (0) 28 9445 1070.

CALIBRATION
0.9% NaCl as zero calibrator and Randox Calibration Serum Level 3 calibrator are recommended for calibration. A 2-point calibration is recommended every 7 days, with change of reagent lot/bottle or as indicated by quality control procedures.

QUALITY CONTROL
Randox Assayed Multi-sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water contaminants, i.e. bacterial growth, may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland + 44 (0) 28 9445 1070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

INTERFERENCE
A Reagent blank may be performed by replacing sample or standard with double deionised water.

The following analytes were tested up to the levels indicated at bile acids concentrations of 21.0 and 94.5 µmol/l and found not to interfere:

	21.0 µmol/l	**94.5 µmol/l**
Haemoglobin:	500 mg/dl	2000 mg/dl
Triglycerides:	500 mg/dl	2000 mg/dl
Intralipid:	2000 mg/dl	2000 mg/dl
Free Bilirubin:	60 mg/dl	60 mg/dl
Conjugated Bilirubin:	60 mg/dl	60 mg/dl

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS
The following performance characteristics were obtained using a RX **daytona** analyser.

LINEARITY
The method is linear up to a concentration of 188 µmol/l.
If the sample concentration exceeds this value, dilute the sample 1+4 with 0.9% NaCl solution and re-assay. Multiply the result by 5.

PRECISION

Intra Assay

	Level 1	Level 2	Level 3
Mean (µmol/l)	12.2	25.81	43.2
SD	0.963	0.868	1.36
CV (%)	7.89	3.36	3.14
n	20	20	20

Inter Assay

	Level 1	Level 2	Level 3
Mean (µmol/l)	13.4	26.0	41.1
SD	1.07	1.31	1.14
CV (%)	7.95	5.03	2.77
n	20	20	20

SENSITIVITY
The minimum detectable level of bile acids with acceptable precision has been determined as 3.20 µmol/l.

METHOD COMPARISON
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.06 X - 0.42
with a correlation coefficient of r = 0.99

40 patient samples were analysed spanning the range 3.0 to 144.5 µmol/l.

REFERENCES
1. Komiyama, Youichi. , Adachi, Tetsuo. , Ito, Yoshimasa., Hikano, Kazuyuki. , Sugiura, Mamoru. , Sawaki, Siiunji. Microassay Of Serum Bile Acids By An Enzymatic Cycling Method, *Chem Pharm Bull* (Tokyo) 30: 3796 – 3797 (1982).
2. Agape, V., Russo, P., Xaiz, L., Calmi, S. , and Grisler, R. Evaluation Of Colorimetric Enzymatic Procedure for Determining The Total Bile Acids In the Blood. *Minerva Dietol Gastroenterol.* Jul-Sep: 35 (3): 159 – 164 (1989).