Bile Acid (Enzymatic)

DiaSystem Scandinavia AB

Diagnostic reagent for quantitative in vitro determination of total bile acids in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size					
01 00041 70 10 0160	R1	4 x 30 mL	+	R2	4 x	10 mL
CDT-TBA	R1	4 x 30 mL	+	R2	4 x	10 mL

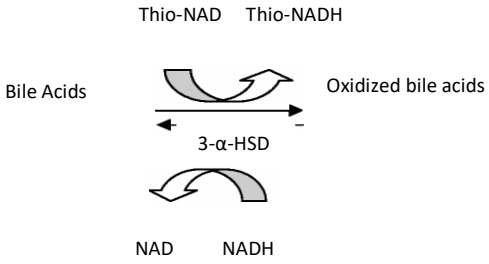
Summary [1,2]

Total bile acids are metabolized in the liver and, hence, serve as a marker for normal liver function. Serum total bile acids are increased in patients with acute hepatitis, chronic hepatitis, liver sclerosis and liver cancer.

Method

The reagents of the assay kit are in a stable liquid formulation that allows for ease of use coupled with enhanced performance characteristics. In the presence of Thio-NAD, the enzyme 3-a-hydroxysteroid dehydrogenase (3- a-HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-a-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 405 nm.

Principle



Reagents

Components and Concentrations

R1:	Thio-NAD	>0.1 mmol/L
	Buffer	
R2:	3-a-HSD	>2 KU/L
	NADH	>0.1 mmol/L
Calibrator		Conjugated cholic acids, Buffer

Storage Instructions and Reagent Stability

Unopened reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C and contamination is avoided. Reagents from different lots must not be interchanged.

Warnings and Precautions

1. For ***in vitro*** diagnostic use.
2. Specimens and reagents containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid swallowing and contact with skin or mucous membranes
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

DiaSystem Bile Acids (Enz.) is ready-to-use. The intrinsic yellow to yellow-brown color of the Total Bile Acids (Enzymatic) does not interfere with the test.

Materials required but not provided

General laboratory equipment

Specimen

Use fresh patient serum samples. Total Bile acid concentration is increased after meals; hence, samples should be collected under fasting conditions. EDTA treated plasma or Lithium heparin plasma samples are suitable for use. Serum or plasma samples are stable for a week at 4 °C, or for 3 months at -20 °C.
Specimens from patients, who are on Ursodeoxycholic Acid (UD-CA) treatment, are not suitable for use with Total Bile Acid (Enzymatic).
Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	Hg 405 nm
Optical path	1 cm
Temperature	37 °C
Measurement	Against water

	Blank	Sample or standard
Sample or standard	-	4 µL
Dist. Water	4 µL	-
Reagent 1	270 µL	270 µL
Mix and incubate for 3 min at 37 °C. Then add:		
Reagent 2	90 µL	90 µL
Mix, read absorbance after 1 min and start stopwatch. Read absorbance again after 2 min.		

Calculation

Calculate ΔA 405/min for sample, blank, and standard by subtracting O.D. value at 60 seconds from O.D. value at 120 seconds.

ΔA 405/min = (O.D. at 120 sec - O.D. at 60 sec)

Determine total bile acids concentration using the equation below:

Sample (TBA, µmol/L) =

$$\frac{\text{Sample } \Delta A405nm/min - \text{Blank } \Delta A405nm/min}{\text{Standard } \Delta A405nm/min - \text{Blank } \Delta A405nm/min} \times \text{Standard}$$

If sample bile acids exceed linear range (1-180 µmol/L), dilute sample with 0.9% NaCl before assay.

Calibrators and Controls

For the calibration of automated photometric systems the DiaSystem UniCal Bile Acid is recommended. The assigned values of the calibrator have been made traceable to a commercially available measurement procedure. For internal quality control DiaSystem UniLab Bile Acid Level 2 and 3 should be assayed with each batch of samples.

	Cat.-no.	Kit size
UniCal Bile Acid	06 00159 70 04 0004	2 x 2 mL
UniLab Bile Acid Level 2 and 3	07 00160 70 04 0001	2 x 1 mL

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Performance characteristics

Measuring range

Linearity studies showed that DiaSystem Bile Acids (Enz.) assay has a linear range from 1 to 180 µmol.
Specimens from patients, who are on Ursodeoxycholic Acid (UDCA) treatment, are not suitable for use with Total Bile Acid (Enzymatic).
A matched set of serum and lithium heparin plasma samples ranging from 0.14-21.18 µmol/L gave a correlation coefficient of 0.9805. Linear regression analysis gave the following equation: Lithium heparin = 0.9972 (serum) + 0.1178 µmol/L.
Samples with a bile acids level exceeding the linearity limit should be diluted with 0.9% saline and re-assayed incorporating the dilution factor in the calculation of the value.

Specificity/Interferences

The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglycerides at 7500 mg/L, Ascorbic acid at 500 mg/L, Bilirubin at 500 mg/L and Hemoglobin at 5000 mg/L.

Sensitivity/Limit of Detection

The lower limit of detection is 1 µmol/L.

Precision

The intra-assay precision and inter-assay precision were evaluated in samples containing two different bile acid levels (8 µmol/L and 23 µmol/L). The inter-assay precision was evaluated by testing these two level specimens (low = 8 µmol/L and high = 23 µmol/L) in 20 runs.

Intra-assay precision n = 20	Mean [µmol/L]	SD [µmol/L]	CV [%]
Level 1 (8µmol/L)	7.93	0.31	3.9
Level 2 (23 µmol/L)	23.50	0.30	1.3

Inter-assay precision n = 20	Mean [µmol/L]	SD [µmol/L]	CV [%]
Level 1 (8 µmol/L)	8.12	0.24	2.9
Level 2 (23 µmol/L)	23.00	0.61	2.6

Method Comparison

A comparison of DiaSystem Bile Acids (Enz.) with a commercially available test using 52 serum samples gave following results:
Fifty-two (52) serum samples ranging from 0.47 - 131.25 µmol/L gave a correlation coefficient of 0.9918. Linear regression analysis gave the following equation:
This method = 1.1536 (reference method) - 0.8567 µmol/L

Reference Range [3]

Serum or plasma containing 0-10 µmol/L bile acids is considered normal range.

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. LaRusso, N.F. et al., Dynamics of Enterohepatic Circulation of Bile Acids, New Engl J M, 291, 689-692, (1974).
2. Skrede S. et al: Bile acids measured in serum during fasting as a test for liver disease, Clin Chem 24: 1095-1099, 1978
3. Wu, Alan H. B. Tietz Clinical Guide to Laboratory Tests. 4th ed. St. Louis, MO: Saunders/Elsevier, 2006. 170-171.

Manufacturer

DiaSystem Scandinavia AB
Datorgatan3, Sweden – 561 33 Jönköping
Phone +46 36 126220 ▪ Fax +46 36 187730
info@diasystem.se ▪ www.diasystem.se

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