



BILE ACIDS

ENZYMATIC COLORIMETRIC DETERMINATION IN SERUM
END POINT, FORMAZAN METHOD

Kit: 10 x 5 mL

Cod. BIL8900

SUMMARY

The major function of the liver is the regulation of the bile acids metabolism, the highest conc. being found in bile. Alterations in bile acid metabolism are usually a reflection of liver dysfunction; cholesterol homeostasis is in large part depending from bile acid metabolism. The combination of non-ionic transport in the small intestine and active transport in the terminal ileum, maintains the bile acid pool.

PRINCIPLE

Bile Acids are converted by 3- α -HSDH (3- α -Hydroxysteroid dehydrogenase) into the corresponding ketons, in presence of NAD. The NADH so formed, reaction with NBT (NitroBlu Tetrazolium) giving a formazan (dye); the reaction is catalyzed by diaphorase. The colour obtained is blue, with a max. absorbance at 540 nm. The intensity of colour at the reaction conditions is directly proportional to the Bile Acids in the sample.

REAGENTS

Components of the kit: **Cod. BIL8900**
***REAGENT 1** (Buffer, liquid, ready to use) **2 x 50 mL**
***REAGENT 2** (Reagent, lyo) **10 x 5 mL**
***REAGENT 3** (Blank, lyo) **10 x 5 mL**
 Phosphate buffer > 10 mmol/L
 3- α -HSDH > 50 U/L
 NAD > 0.1 mmol/L
 NBT > 0.1 mmol/L
 Diaphorase > 200 U/L
***REAGENT 4** (Std 5 μ mol/L, liq., ready to use) **1 x 6 mL**
***REAGENT 5** (Std 25 μ mol/L, liq., ready to use) **1 x 6 mL**
***REAGENT 6** (Std 100 μ mol/L, liq., ready to use) **1 x 6 mL**

STABILITY: the reagents, stored at 2-8°C, are stable up to the expiration date shown on the package **if not contaminated during handling.**

PREP. OF THE *WORKING REAGENT (*R1+*R2)

Dissolve a vial of *Reagent 2 with 5 ml of *Reagent 1. Mix gently until dissolution. Please avoid foam formation.

Mix Kindly and let the working reagent reach the working temperature before use. Close immediatly after handling. Incompetent handling will keep us harmless from any responsibility.

STABILITY: 7 days at 2-8°C.

PREP. OF THE *BLANK REAGENT (*R1+*R3)

Dissolve a vial of *Reagent 3 with 5 ml of *Reagent 1. Mix gently until dissolution. Please avoid foam formation.

Mix Kindly and let the working reagent reaches the working temperature before use. Close immediatly after handling. Incompetent handling will keep us harmless from any responsibility.

STABILITY: 7 days at 2-8°C.

SAMPLE

- No haemolyzed serum

PROCEDURE

- Wavelength: 540 nm (520-550 nm)
- Pathlength: 1 cm
- Reading: against Blank Reagent
- Temperature: 37°C
- Method: end-point
- Reaction: 15 minuti
- Linearity: up to 200 μ mol/L
- Sample/reagents: 1,0 / 2,5

Let the reagent reach the working temperature before use.

PROCEDURE NOTE

For the One point Calibration, use *Reagent 6 (Standard 100 μ mol/L).

Pipett in 4 test tubes or cuvettes so labelled:

S/B: Sample Blank, S: Sample, St/B: Standard Blank, St: Standard;

	S/B	S	St/B	St
*Working Reagent	---	1000 μl	---	1000 μl
Sample	400 μ l	400 μl	---	---
*Blank Reagent	1000 μ l	---	1000 μ l	---
*Reagent 6 Std. 100 μ mol/L	---	---	400 μ l	400 μl

Mix kindly and incubate for about 15 min. at 37°C.

Read the absorbance of Sample (AS), of sample Blank (ASB), of Standard (Ast) and Standard Blank (AstB).

The colour is stable only 30 min. at room temperature and in the dark.

NOTE

ONLY in the extreme need to have a colour stable at least 24 h at 2-8°C in the dark, is necessary to block the enzymatic reactions, adding a diluted acid solution (HCl 0,2N).

Add to the upper cuvettes following volumes of Diluted Acid (NOT SUPPLIED), who could be prepared with deep attention and with due and right safety protections

- dilute 2 mL of HCl 32% to a 100 mL with bid. water; or
- dilute 10 mL of HCl 2N to 100 mL with bid. water; or
- Dilute 20 ml of HCl 1N to 100 mL with bid. water.

Hydrochloric acid 0,2N (HCl 0,2N)	500 μ l	500 μ l	500 μ l	500 μ l
---	-------------	-------------	-------------	-------------

Mix gently with deep attention; read the absorbance as upper described.

CALCULATION for One Point Calibration

Calculate $\Delta AS = AS - ASB.$

Calculate $\Delta Ast = Ast - AstB. (Std 100 \mu\text{mol/L})$

$(\Delta AS / \Delta Ast) \times 100 = \text{Bile Acid Concentration in } \mu\text{mol/L}$

CALCULATION FOR Curve of Calibration

Calculate $\Delta AS = AS - ASB.$

Calculate $\Delta Ast = Ast - AstB.$

(using the Standard 5 - 25 - 100 $\mu\text{mol/L}$)

Plotting the Standards Absorbances against the concentrations, may be obtained the Calibration Curve; referring to it the Samples Absorbances, may be calculate the Samples Concentrations.

REFERENCE VALUES

Bile Acids: till 8,1 $\mu\text{mol/L}.$

It is suitable that every laboratory determines its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The Bile Acids Reagent is linear up to 200 µmol/L. For concentrations ≥ 200 µmol/L, dilute the sample 1:4 with saline sol., repeat the determ. and multiply the result $\times 4$.

Sensitivity: An absorbance change of about 0,004 corresponds to a Bile Acids conc. of 1 µmol/L.

Within-run Precision:

	Mean (µmol/L) \pm 2s	CV %
Serum 1	5,02 \pm 0,48	4,77
Serum 2	11,98 \pm 0,69	2,86

Run-to-run (Day-to-day) Precision:

	Mean (µmol/L) \pm 2s	CV %
Serum 1	4,99 \pm 0,62	6,21
Serum 2	12,00 \pm 0,84	3,51

Accuracy: with commercially available Control(s)

	Waited (µmol/L)	Found (µmol/L)
	25,3 (20,2- 30,4)	25,6-26,2-26,7

Interferences: See References point 2.

Correlation: A group of 20 sera from 3,5 to 117µmol/L was assayed by this procedure and using a similar commercially available Bile Acids Reagent. Comparison of the data gave following results:

Linear regression equation $y = 0,9930x + 0,19$
Correlation coefficient $r = 0,9983$

NOTE

1. A proportional. variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concentrations of Bile Acids ≥ 200 µmol/L, dilute the sample 1:4) with saline sol., repeat the determination and multiply the result $\times 4$.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Bile Acids (see References 2.).
5. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).
3. Mashige F. et al., Clin. Chem. 24, 1150 (1978).

Ver. 12/03