BILE ACIDS LR liquid reagent

REF 1100140
R1 1x30 + R2 1x10 +
CAL 1x2 ml

CE IVD For in vitro medical device

Use
Total Bile Acid Assay kit is intended for the in vitro quantitative determination of serum total bile acids (TBA).

Summary
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.

Principle
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-α-hydroxysteroid dehydrogenase (3-α-HSD) converts bile acids to 3-keto steroids and Thio-NADH.
The reaction is reversible and 3-α-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.

Reagents
R1 Thio-NAD, Buffer > 0.1 mM
R2 3-α-HSD, >2kU/L ; NADH>0.1 mM Buffer
Calibrator Conjugated cholic acids, Buffer

Reagents Preparation
Reagents are liquid and ready to use. Keep out the reagents from refrigerator only for the use and recap them immediately.

Storage And Stability
Store the kit at 2-8°C.
Unopened reagents are stable until the expiration date printed on the label.
Reagents from different lots must not be interchanged.

Precaution in Use
The product is not classified as dangerous (DLg. The final concentration of the components is below the limits imposed by Regulation (EC) No. 1272/2008 - CLP (and subsequent amendments) and Directive 88/379/CEE and subsequent amendments to the classification-packaging and labeling of dangerous substances N. 285 art. 28 l. n. 128/1998).
However the reagent should be handled with care, according to good laboratory practice.
Caution: the reagents contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contacting with skin, eyes and mucous membranes. In case of contact with eyes rinse immediately with plenty of water and seek medical advice.

Waste Management
Please refer to the local legal requirements.

Specimen Collection and Preparation
Use fresh patient serum sample. TBA 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.

Note
- The kit, according to this method, must be used in manual procedures. About automatic using follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the singles vials.

Procedures

Wavelength	λ: 405 nm
Working temperature	37°C
Optical path	1 cm
Reaction	Fixed Time

Bring the reagents at 15-25°C before using them.

Procedure

	STD	SAMPLE
Reagent R1	270 µl	270 µl
Sample	--	4 µl
Standard	4 µl	--

Mix, incubate at 37°C for 5' and then add:

	STD	SAMPLE
Reagent R2	90 µl	90 µl

Mix, then incubate at 37°C. Measure the absorbance values of first reading after 60" from sample adding (E1C) and standard (E1STD). Read a second time after 60" (E2C), (E2STD).

Calculation
TBA [µEq/L] o [µmol/l] =
(E2C - E1C) / (E2STD - E1STD) x Conc. STD

Reference Values to 37°C
Serum - plasma 0-10 µmol/l

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

ANALYTICAL PERFORMANCES

Analytical Sensitivity and Linearity
Total Bile Acids assay has a linear range from 0 to 180 µmol/l.
Samples with values exceeding this range must be diluted with saline solution. Then multiply the result for the diluting factor.

"Intra-Assay" precision (within-Run)
Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (U/l)	N = 7.93	H = 23.5
S.D.	N = 0.31	H = 0.3
C.V.%	N = 3.9	H = 1.3

"Inter-Assay" precision (between-run)
Determined on 20 samples for each control (N-H) Results:

MEAN (U/l)	N = 8.12	H = 23.0
S.D.	N = 0.24	H = 0.61
C.V.%	N = 2.9	H = 2.6

Correlation
A study based comparing this method with a similar method on 20 samples has given a correlating factor **r = 0.9805**

y = 0.9972 x + 0.1178

Interferences
No interferences was observed by the presence of :

Bilirubin	≤ 50 mg/dl
Triglycerides	≤ 750 mg/dl
Ascorbate acid	≤ 50 mg/dl
Hemoglobin	≤ 500 mg/dl

Quality Controls
It's necessary, each time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should estabilish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography
Goldstein, D.E.et al, Diabetes Care. 27(7).1761-73(2004)
United Kingdom Prospective study;1998; Lancet 352:837-53

Symbols

CE	CE Mark (98/79 CE regulation)
IVD	in vitro medical device
LOT	Batch Code
	Use by
	Storage temperature limits
	Read instruction for use
	Gesan Production srl