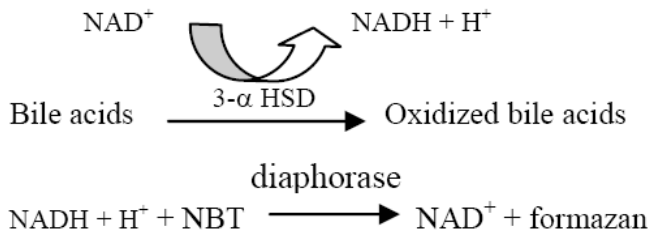
Total Bile Acids Assay Kit (Colorimetric)
Catalog Number: BQ 092A-EALD

Intended Use
The assay kit is for the determination of serum total bile acids (TBA). For investigational use or export only.

Clinical Significance
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.

Assay Principle
In the presence of NAD, the enzyme 3-α hydroxysteroid dehydrogenase (3-α HSD) converts bile acids to 3-keto steroids and NADH. The NADH formed reacts with nitrotetrazolium blue (NBT) to form a formazan dye in the presence of diaphorase enzyme. The dye formation is monitored by measuring absorbance at 540nm and is directly proportional to the bile acids concentration in the serum sample.



Reagent Composition

Reconstitution Buffer (R1)	Phosphate buffer, EDTA	1 x 105 mL
Reagent 2 (R2)	3-α-HSD, Tris buffer	1x 20 mL
Reagent 3 (R3)	Diaphorase, NAD^+, NBT, Oxamic Acid	10 x 10 mL freeze-dried powder
Bile Acids Standard	35µmole/L	2 mL

Materials Required but not Provided
An analyzer capable of dispensing two reagents and of measuring absorbance at about 540nm with temperature control (37 °C).

Controls for validating the performance of the bile acid reagents are provided separately.

Reagent Preparation
Transfer 10mL of the contents of diluent R1 to one bottle of R3 (diaphorase) and dissolve by swirling gently. Reconstituted R3 is stable for 1 week at 4 °C.

Reagent Stability and Storage
The colorimetric total bile acids assay kit, calibrators, and controls should be stored at 2-8 °C. DO NOT FREEZE. The reagents, calibrators, and controls are stable when stored as instructed until the expiration date on the label. Do not mix reagents of different lots.

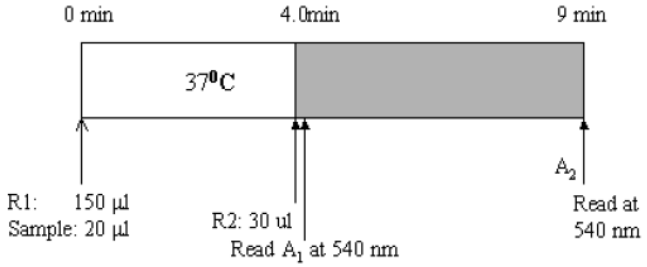
The reconstituted R3 is stable for 1 week at 4 °C.

Specimen Collection and Handling
Use fresh patient serum or EDTA treated plasma samples. Hemolysed or heparinized samples should not be used.

Assay Procedure
1. Reconstitute the contents of one bottle of R3 (diaphorase) with 10 mL of reconstitution buffer R1. Reconstituted R3 is stable for 1 week at 4 °C.
2. Pre-warm reconstituted R1 and R2 at RT.
3. To a cuvette add 150 µl of reconstituted R3 and 20 µl of sample or standard, mix well, and incubate at 37 °C for 4 min.
4. Add 30 µl of R2, mix well, and immediately read the absorbance at 540 nm as A_1.
5. Incubate for 5 min, and read the absorbance at 540 nm as A_2.
6. Calculate ΔA540/5min for sample and standard by subtracting A_1 from A_2. ΔA540/5min = (A_2 - A_1).
7. Determine total bile acids concentration using the equation below:

Sample Bile Acids (µmole/L) =

$$\frac{\Delta A_{540sample}}{\Delta A_{540standard}} \times \text{standard (35µmole/L)}$$



Calibration
A single level of calibrator included are ready to use and are stable up to expiration date when stored at 2-8 °C.

1. This assay should be calibrated daily using the enclosed calibrator.
2. Construct a calibration curve by plotting the ΔA values of the calibrators against the corresponding concentrations.
3. The bile acid concentration of the samples is read from the calibrations curve.

A reagent blank may be performed by replacing samples or standard with distilled water.

Quality Control
Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state, and local guideline concerning the running of external quality control.

To ensure adequate quality control, normal and abnormal control with known values should be run as unknown samples.

Results
Results are printed out in µmole/L.

Reference Range
Serum or plasma 0-10 µmole/L is considered normal range.

Limitations
The assay is designed for use with fresh serum sample and EDTA treated plasma only.
Linearity is up to 200 µmole/L. Samples that exceed the linearity limit should be diluted with an equal volume of 0.9% saline. Multiply the result by two.

Linearity
The method is linear up to a concentration of 200 µmole/L. Samples above this concentration should be diluted with 0.9% saline (0.15 M NaCl).

Safety Precautions
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions 1 and 2 contain sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water.

Sodium azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volume of water to prevent azide build up.

Avoid use of haemolyzed samples and heparinized plasma as these interfere with the assay.

Hitachi 717 Parameters
Temperature 37 °C

Use the following parameters with calibrator for calibration.

Test	CTBA
Assay Code	2 Point
Assay Point	(26)-(49)
Wavelength	700/546
Calibration Method	Linear
Unit	µmol/L
Sample volume	(20)(20)
Reagent vol. R1	(150)(100)(NO)
Reagent vol. R2	(30)(100)(NO)
STD (1) CONC.-POS	(0)-(1)*
STD (2) CONC.-POS	(35)-(2)*
ABS.Limit	32000-Increase
Expected value (normal Value)	0-10
Tech Limit	0-180
Standard position	*
Standard Conc.	35.0
Water position	*
Water Conc.	0.0

Attention: * Entered by Operator
** Each reading cycle is 12 seconds

** The above reagent parameter **has not been fully validated** for these analyzers. The parameters are based on Bio-Quant's knowledge of the analyzer and reagents, and should perform adequately. However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.

Hitachi 917 Parameters
Temperature 37 ºC

Use the following parameters with calibrator for calibration.

Test	CTBA
Assay Code	2 Point End
Assay Point	(10)(18)(34)(0)(0)
Wavelength	700/546
Sample volume (normal)	(20)(0)(0)
Sample volume (Dec.)	(20)(0)(0)
Sample volume (Inc.)	(20)(0)(0)
Diluent	(water)(0)
Reagent vol. R1	(150)(0)(10015)(0)
Reagent vol. R2	(0)(0)(10015)(0)
Reagent vol. R1	(30)(0)(10015)(0)
Reagent vol. R1	(0)(0)(10015)(0)
ABS.Limit	32000-Increase
STD (1) CONC	0
POS.	*
STD (2) CONC	35
POS.	*

Attention: * Entered by Operator
** Each reading cycle is 18 seconds

Cobas Mira Parameters
Temperature 37 °C

Use the following parameters with calibrator for calibration.

Measurement mode	Absorb	offset	0.0000
Reaction Mode		R-S-SR1	
Calibration Mode	Slope Avg.	Test range low	0.0000 umol/L
Reagent Blank	Reag/DIL	Test range High	200.00 umo1/L
Cleaner		No	
Wavelength	500 nm	Number of steps	1
Decimal position	3	Calc. Step A	Endpoint
Unit		Umol/l	
Sample cycle	1	Readings first	11
Sample volume	20.0 uL	Reading last	23
Sample dilution		H_2O	
Dilution volume	0.0 uL		Calibration
Cali. Interval		Each day	
Reagent cycle	1	Time	No
Reagent volume		150 uL	
Dilution volume	0.0 uL		Blank
Reagent range low		0.0	
Start R1 cycle	10	High	0.8
Reagent volume	30 uL	Blank Range low	-0.1
Dilution volume	0.0 uL	High	0.1
Sample limit	No	Standard pos	1
Reaction direction	Increase	Standard -1	35.0umol/l
Convers. Factor	1.0000	Replicate	Dupl
Replicate		Dupl	

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