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

**Intended Use**

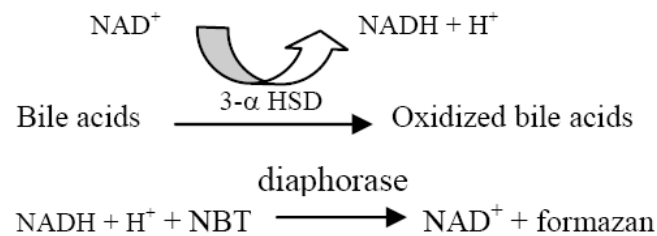
The assay kit is for the determination of serum total bile acids (TBA). For investigational use or export only.

**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- $\alpha$  hydroxysteroid dehydrogenase (3- $\alpha$  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- $\alpha$ -HSD, Tris buffer	1x 20 mL
Reagent 3 (R3)	Diaphorase, NAD <sup>+</sup> , NBT, Oxamic Acid	10 x 10 mL freeze-dried powder
Bile Acids Standard	35 $\mu$ mole/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**

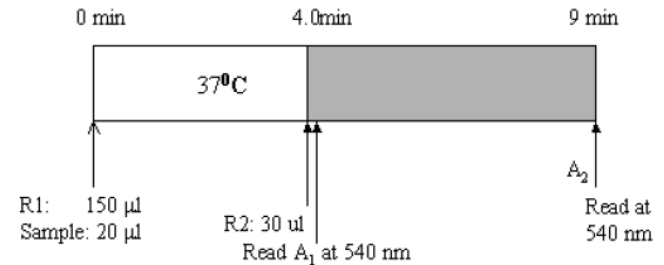
Use fresh patient serum or EDTA treated plasma samples. Hemolysed or heparinized samples should not be used.

**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  $\mu$ l of reconstituted R3 and 20  $\mu$ l of sample or standard, mix well, and incubate at 37 °C for 4 min.
4. Add 30  $\mu$ l of R2, mix well, and immediately read the absorbance at 540 nm as A<sub>1</sub>.
5. Incubate for 5 min, and read the absorbance at 540 nm as A<sub>2</sub>.
6. Calculate  $\Delta A_{540/5\text{min}}$  for sample and standard by subtracting A<sub>1</sub> from A<sub>2</sub>.  $\Delta A_{540/5\text{min}} = (A_2 - A_1)$ .
7. Determine total bile acids concentration using the equation below:

Sample Bile Acids ( $\mu$ mole/L) =

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



**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  $\Delta A$  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  $\mu$ mole/L.

**Reference Range**

Serum or plasma 0-10  $\mu$ mole/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  $\mu$ mole/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 °C

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 µmol/L
Reagent Blank	Reag/DIL	Test range High	200.00 µmol/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 <sub>2</sub> O		
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	35.0µmol/l
Convers. Factor	1.0000	Replicate	Dupl
Replicate	Dupl		

\*\* The above reagent parameters **have 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.