



## Colorimetric Total Bile Acids Assay Kit

### Configuration

The Diazyme Colorimetric Total Bile Acids reagent is provided in the following kit configuration:

Instrument	Catalog No.	Kit Size
Universal	DZ092A-K	R1: 1 x 105 mL* R2: 1 x 20 mL R3: 10 x 10 mL Cal: 1 x 2 mL *diluent

### Intended Use

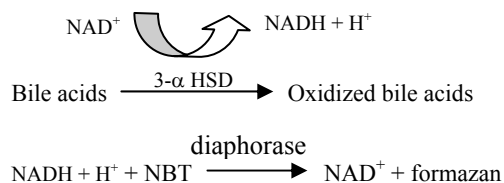
The assay kit is for determination of serum total bile acids (TBA). For investigational use or export only.

### 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 nitrotriazolium 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.



### Materials Required but not Provided

An analyzer capable of dispensing two reagents and of measuring absorbance at about 540 nm with temperature control (37°C).

Controls for validating the performance of the bile acid reagents are provided separately (DZ092A-Con).

### Reagent Composition

	Active Ingredients
Reconstitution buffer (R1)	Phosphate buffer, EDTA
Reagent 2	3- $\alpha$ -HSD, Tris buffer
Reagent 3	Diaphorase, NAD <sup>+</sup> , NBT, Oxamic Acid
Bile Acids Standard	35 $\mu$ mol/L

### Reagent Preparation

Transfer 10 mL 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

Diazyme 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.

### 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 haemolyzed samples and heparinized plasma as these interfere with the assay.

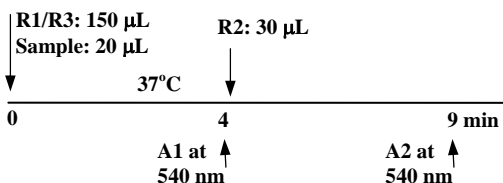
Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

### 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<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 (µmole/L) =

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



### 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  $\Delta A$  values of the calibrators against the corresponding concentrations.
3. The bile acid concentration of the sample is read from the calibration curve.

A Reagent blank may be performed by replacing sample 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 µmol/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 exceeded 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 µmol/L. Samples above this concentration should be diluted with 0.9% saline (0.15 M NaCl).