Colorimetric Total Bile Acids Assay Kit

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

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Catalog No.</th>
<th>Kit Size</th>
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</thead>
<tbody>
<tr>
<td>Universal</td>
<td>DZ092A-K</td>
<td>R1 (diluent): 1 x 105 mL&lt;br&gt;R2: 1 x 20 mL&lt;br&gt;R3: 10 x 10 mL&lt;br&gt;Cal: 1 x 2 mL</td>
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**Intended Use**
The assay kit is for determination of serum total bile acids (TBA).

**Background**
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 research subjects 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.

\[
\text{NAD}^+ \xrightarrow{3-\alpha \text{HSD}} \text{NADH} + \text{H}^+ \\
\text{Bile acids} \xrightarrow{3-\alpha \text{HSD}} \text{Oxidized bile acids} \\
\text{diaphorase} \\
\text{NADH} + \text{H}^+ + \text{NBT} \xrightarrow{\text{diaphorase}} \text{NAD}^+ + \text{formazan}
\]

**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**

<table>
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<th>Active Ingredients</th>
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<td>Reconstitution buffer (R1)</td>
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<td>Phosphate buffer, EDTA</td>
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</table>

| Reagent 2 | 3-α-HSD, Tris buffer |
| Reagent 3 | Diaphorase, NAD⁺, NBT, Oxamic Acid |

**Bile Acids Standard**
35 µmole/L

**Reagent Preparation**
Transfer 10 mL of the contents of diluent R1 to one bottle of R3 (diaphorase) and dissolve by swirling gently.

**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.

- R3 is light and temperature sensitive. R3 should not be stored in temperatures above refrigerated conditions (2-8°C) or exposed to light for extended periods of time.

The Reconstituted R3 is stable for 1 week at 4°C

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

**Precautions**
1. For Research Use Only in the USA. Not for use in diagnostic procedures.
2. Avoid use haemolyzed samples and heparinized plasma as these interfere with the assay.
3. Inspect R3 before use. Do not use R3 if it is a dark color.
4. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
5. 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.
6. 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.
7. 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 cus-
tomer 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 A1.

5. Incubate for 5 min, and read the absorbance at 540 nm as A2.


7. Determine total bile acids concentration using the equation below:

\[
\text{Sample Bile Acids (μmole/L)} = \frac{\Delta A_{540\text{sample}}}{\Delta A_{540\text{standard}}} \times \text{standard (35μmole/L)}
\]

**Calibration**

A single level of calibrator included is 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 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. Literature reports cite subjects having a TBA activity in the range of 0-10μmol/L\[1\]².

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**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.

**References**
