Total bile acids 21 FS*

Order Information
Cat. No. 1 2238 99 10 930
Kit size R1 4 x 12 mL + R2 2 x 8 mL

Intended Use
Reagent for quantitative in vitro determination of total bile acids in serum on photometric systems

Summary
Bile acids (BA) are water soluble, amphipathic end products of the cholesterol catabolism which are synthesized in the liver, stored in the gall bladder and secreted in the intestine during digestion [1,2]. Throughout this metabolism BA change their form from primary via secondary to tertiary BA and their conjugates. Total bile acids (TBA) refer to the sum of all these forms. Serum TBA levels are a sensitive marker of liver function, reflecting hepatic synthesis, secretion and re-absorption [2,3]. Compared to conventional liver screening tests such as ALT or AST, which indicate acute liver damage, the determination of total bile acids allows early detection of liver dysfunction and early treatment and prevention of severe, irreversible liver damage. Once a patient suffers from a liver disease, serum TBA can be used to monitor the treatment response [4-6]. Although TBA levels provide early diagnosis of hepatobiliary deficiencies they do not allow the differentiation between various diseases. Increased serum TBA levels are associated with several diseases such as acute and chronic hepatitis, intrahepatic cholestasis of pregnancy (ICP), liver sclerosis, cirrhosis, and cancer [2-9]. The determination of TBA levels in pregnant women is considered to be the most important biomarker for diagnosis and monitoring of ICP, also known as obstetric cholestasis [10-12]. ICP is the most common liver disease that occurs during pregnancy; usually during the last 3 months of pregnancy. It is caused by a reversible, hormonally bile secretion disturbance which leads to a restricted bile flow through the gallbladder and in turn, to an accumulation of bile acids in the liver and possibly in the bloodstream [7,13]. Pregnancy-cholestasis is characterized by strong itching (pruritus) [11]. During ICP, TBA levels may rise up to 220 µmol/L [12], leading to an increased risk of fetal distress, premature birth or even stillbirth. TBA concentrations above 40 µmol/L may be fetotoxic [11]. Decreased serum TBA levels are associated with fidal function, malabsorption, diarrhea or Crohn’s disease. In the veterinary field serum TBA measurements are also of common practice. [1-12]

Method
Enzymatic cycling method
Two reactions are combined in the new generation enzymatic cycling method. In the presence of Thio-NAD, the enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD) converts bile acids to 3-ketosteroids and Thio-NADH. The reaction is reversible and 3α-HSD can convert 3-ketosteroids and NADH to bile acids and NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring the specific change of absorbance at 405 nm. This cycling reaction leads to significant signal amplification. [14]

Reagents
Components and Concentrations
R1: Buffer Thio-NAD > 0.1 mmol/L
R2: Buffer 3α-HSD > 2 KU/L

Storage and Reagent Stability
The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents and protect them from light.

Warnings and Precautions
1. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Postprandial serum TBA levels are generally higher than fasting serum TBA levels. Thus, fasting samples should be used for bile acid determination [3].
3. In very rare cases, samples of patients with gammopathy might give falsified results [15].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
5. For professional use only.

Waste Management
Refer to local legal requirements.

Reagent Preparation
The reagent is ready to use.

Materials Required
General laboratory equipment

Specimen
Serum (fasting > 12h)
Samples from patients under bile acid analogues treatment such as fusidic acid, ursodeoxycholic acid or obeticholic acid are unsuitable for analysis [19].

Stability:
1 day at 20 – 25°C
1 week at 2 – 8°C
1 year at –20°C

Only freeze once. Discard contaminated specimens.

Assay Procedure
Applications for automated systems are available on request.

Wavelength 405 nm / 600 nm (bichromatic)
Optical path 1 cm
Temperature 37°C
Measurement Against reagent blank

<table>
<thead>
<tr>
<th>Sample/Calibrator</th>
<th>Blank</th>
<th>Sample/ Calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dist. Water</td>
<td>4 µL</td>
<td>4 µL</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>270 µL 270 µL</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>90 µL 90 µL</td>
<td></td>
</tr>
</tbody>
</table>

Mix and incubate 5 min. at 37°C. Then add:
Mix and read absorbance after 1 minute and start stop watch. Read absorbance again after 1 and 2 minutes.

ΔA/min. = (ΔA/min. sample/calibrator – ΔA/min reagent blank)
Calculation
With calibrator

\[ \text{Bile acids [µmol/L]} = \frac{\Delta A/\text{min. Sample} \times \text{Conc. Cal [µmol/L]}}{\Delta A/\text{min. Cal}} \]

Calibrators and Controls
DiaSys TruCal TBA calibrator is recommended for calibration. The assigned values of the calibrator have been made traceable to a commercially available measurement procedure. Use Diasys TruLab N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
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</thead>
<tbody>
<tr>
<td>TruCal TBA</td>
<td>1 2240 99 10 037 x 1 mL</td>
</tr>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 061 x 6 mL</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 062 x 6 mL</td>
</tr>
</tbody>
</table>

Performance Characteristics
Data evaluated on BioMajesty® JCA-BM6010/C
Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

- Measuring range up to 220 µmol/L.
- When values exceed this range samples should be diluted 1 + 5 with NaCl solution (9 g/L) and the result multiplied by 6.
- Limit of detection** = 2 µmol/L

<table>
<thead>
<tr>
<th>Interfering substance</th>
<th>Interferences ≤ 10% up to Total bile acids [µmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>100 mg/dL</td>
</tr>
<tr>
<td>Bilirubin conjugated</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Bilirubin unconjugated</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Lipemia (Triglycerides)</td>
<td>700 mg/dL</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>350 mg/L</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>350 mg/L</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>30 mg/L</td>
</tr>
</tbody>
</table>

For further information on interfering substances refer to Young DS. [16]

Reference Range
< 10 µmol/L (fasting)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

* Fluid Stable