ALAT (GPT) FSC* (IFCC mod.)
with/without pyridoxal-5-phosphate

Concentrated diagnostic reagent for quantitative in vitro determination of ALAT (GPT) in serum or plasma on Olympus AU systems

Order information
Cat. No. Kit size
2 2705 99 10 440 R1 3 x 60 mL + R2 3 x 45 mL
2 5010 99 10 030 6 x 3 mL

Summary [1,2]
Alanine Aminotransferase (ALAT/ALT), formerly called Glutamic Pyruvic Transaminase (GPT) and Aspartate Aminotransferase (ASAT/AST), formerly called Glutamic Oxalacetic Transaminase (GOT) are the most important representatives of a group of enzymes, the aminotransferases or transaminases, which catalyze the conversion of α-keto acids into amino acids by transfer of amino groups. As a liver specific enzyme ALAT is only significantly elevated in hepatobiliary diseases. Increased ASAT levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma. Parallel measurement of ALAT and ASAT is therefore applied to distinguish liver from heart or skeletal muscle damages. The ASAT/ALAT ratio is used for differential diagnosis in liver diseases. While ratios < 1 indicate mild liver damage, ratios >1 are associated with severe, often chronic liver diseases.

Method
Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)[modif.].

Principle
L-Alanine + 2-Oxoglutarate \( \xrightarrow{\text{ALAT}} \) L-Glutamate + Pyruvate
Pyruvate + NADH + H⁺ \( \xrightarrow{\text{LDH}} \) D-Lactate + NAD⁺

The rate of the decrease of NADH is measured photometrically and is direct proportional to the ALAT-activity in the specimen.

Addition of pyridoxal-5-phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, e.g. from patients with myocardial infarction, liver disease and intensive care patients [1].

Reagents
Components and Concentrations
R1: TRIS pH 7.5 100 mmol/L
L-Alanine 500 mmol/L
Lactate dehydrogenase (LDH) ≥ 1.7 kU/L
R2: 2-Oxoglutarate ≥ 15 mmol/L
NADH 0.18 mmol/L
Pyridoxal-5-Phosphate FS
Good’s buffer pH 9.6 0.7 mmol/L
Pyridoxal-5-phosphate 0.1 mmol/L

Components and Concentrations in the test mixture
R1: TRIS pH 7.5 680 mmol/L
L-Alanine 3.2 mol/L
Lactate dehydrogenase (LDH) ≥ 8 kU/L
R2: 2-Oxoglutarate 140 mmol/L
NADH 2.5 mmol/L
Pyridoxal-5-Phosphate FS
Good’s buffer pH 9.6 100 mmol/L
Pyridoxal-5-phosphate 13.8 mmol/L

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

Warnings and Precautions
1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Take the necessary precautions for the use of laboratory reagents.

Waste Management
Please refer to local legal requirements.

Reagent Preparation
The reagents are ready to use.
The bottles are placed directly into the rotor of the respective analyzer.
For the determination with pyridoxal-5-phosphate (P-5-P) mix 20 mL of reagent 1 with 1 mL of P-5-P.

Materials required but not provided
DiaSys Pyridoxal-5-Phosphate FS in case of determination with P-5-P activation (Cat.No. 2 5010 99 10 030)
NaCl solution 9 g/L
General laboratory equipment

Specimen
Serum, heparin plasma or EDTA plasma
Stability [4]:
3 days at 20 – 25 °C
7 days at 4 - 8 °C
7 days at -20 °C
Discard contaminated specimens.

Assay Procedure
The assay procedure and the calculation refer to the manual of the analyzing system and the appropriate application.
Calibrators and Controls
For the calibration of automated photometric systems the DiaSys TruCal U calibrator is recommended. For internal quality control DiaSys TruLab N and P controls should be assayed with each batch of samples.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCal U</td>
<td>5 9100 99 10 063</td>
</tr>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 062</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 062</td>
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</tbody>
</table>

Performance Characteristics
Measuring range
The test has been developed to determine ALAT activities within a measuring range from 2 – 800 U/L. If such value is exceeded, the sample should be diluted 1+9 with NaCl solution (9 g/l) and results multiplied by 10.

Specificity/Interferences
No interference was observed by bilirubin up to 30 mg/dL, hemoglobin up to 400 mg/dL and lipemia up to 1,000 mg/dl triglycerides.

Sensitivity/Limit of Detection
The lower limit of detection is 2 U/L.

Precision (with pyridoxal–5-phosphate)

<table>
<thead>
<tr>
<th>Intra-assay precision</th>
<th>n = 20</th>
<th>Mean [U/L]</th>
<th>SD [U/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>36.5</td>
<td>2.34</td>
<td>6.43</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>77.8</td>
<td>1.29</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>117.4</td>
<td>1.94</td>
<td>1.66</td>
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</table>

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<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>35.4</td>
<td>1.02</td>
<td>5.15</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>73.3</td>
<td>2.99</td>
<td>4.08</td>
<td></td>
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<tr>
<td>Sample 3</td>
<td>120.0</td>
<td>2.38</td>
<td>1.99</td>
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Method Comparison
A comparison between DiaSys ALAT (GPT) FSC with P-5-P (y) and a commercially available test (x) using 97 samples gave following results:
\[ y = 1.021 x - 2.626 \text{ U/L}; \ r = 0.998. \]

Reference Range

**With pyridoxal-5-phosphate activation**

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Women [3]</td>
<td>&lt; 34 U/L</td>
</tr>
<tr>
<td>Men [3]</td>
<td>&lt; 45 U/L</td>
</tr>
<tr>
<td>Children [1]</td>
<td>1 – 30 days</td>
</tr>
<tr>
<td></td>
<td>2 – 12 months</td>
</tr>
<tr>
<td></td>
<td>1 – 3 years</td>
</tr>
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<td></td>
<td>4 – 6 years</td>
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<td></td>
<td>7 – 9 years</td>
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<tr>
<td></td>
<td>10 – 18 years</td>
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</table>

**Without pyridoxal-5-phosphate activation**

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<table>
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</thead>
<tbody>
<tr>
<td>Women</td>
<td>&lt; 31 U/L</td>
</tr>
<tr>
<td>Men</td>
<td>&lt; 41 U/L</td>
</tr>
</tbody>
</table>

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

Literature

Manufacturer
DiaSys Diagnostic Systems GmbH
Alter Strasse 9 65558 Holzheim  Germany