# ALT (GPT) IFCC mod

DiaSystem Scandinavia AB

**with/without Pyridoxalphosphate**

**Diagnostic reagent for quantitative in vitro determination of ALAT (GPT) in serum or plasma on photometric systems**

## Order Information

| Cat. No. | Kit size | | | | | |
|---|---|---|---|---|---|---|
| 01 00001 70 04 0125 | R1 | 5 x | 20 mL | + R2 | 1 x | 25 mL |
| 01 00001 70 04 0500 | R1 | 5x | 80 mL | + R2 | 1 x | 100 mL |
| 01 00001 70 10 0180 | R1 | 4 x | 36 mL | + R2 | 4 x | 9 mL |
| 01 00001 70 02 0240 | R1 | 4 x | 36 mL | + R2 | 4 x | 9 mL |
| CDT-ALT | R1 | 3x | 30 mL | + R2 | 2x | 11,3 mL |

For determination with Pyridoxalphosphate activation additionally required:

| | | |
|---|---|---|
| 01 00120 70 04 0018 | 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)[modified].

## Principle

L-Alanine + 2-Oxoglutarate <—ALAT—> L-Glutamate + Pyruvate

Pyruvate + NADH + $H^+$ <—LDH—> D-Lactate + $NAD^+$

Addition of pyridoxal-5-phosphate (P-5-P), recommended by IFCC stabilizes the activity of 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, 3].

## Reagents

**Components and Concentrations**

| | | | |
|---|---|---|---|
| **R1:** | TRIS | pH 7.15 | 140 mmol/L |
| | L-Alanine | | 700 mmol/L |
| | LDH (lactate dehydrogenase) | | ≥ 2300 U/L |
| **R2:** | 2-Oxoglutarate | | 85 mmol/L |
| | NADH | | 1 mmol/L |
| **Pyridoxalphosphate** | | | |
| | Good's buffer | pH 9.6 | 100 mmol/L |
| | Pyridoxal-5-phosphate | | 13 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. Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. Sulfasalazine and sulfapyridine medication may lead to false results in patient samples. Blood collection must be done before drug administration.
5. 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.
6. For professional use only!

**Waste Management**

Please refer to local legal requirements.

**Reagent Preparation**

***Substrate Start***

The reagents are ready to use.
For the determination with Pyridoxalphosphate (P-5-P) mix 1 part of P-5-P with 100 parts of reagent 1,
e.g. 100 µL P-5-P + 10 mL R1

| | | | |
|---|---|---|---|
| Stability after mixing: | 6 days | at | 2 - 8 °C |
| | 24 hours | at | 15 - 25 °C |

***Sample Start***

*without Pyridoxalphosphate*

Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono reagent

| | | | |
|---|---|---|---|
| Stability: | 4 weeks | at | 2 - 8° C |
| | 5 days | at | 15 - 25° C |

The mono reagent must be protected from light!

**Materials required but not provided**

DiaSystem Pyridoxalphosphate in case of determination with P-5-P activation (Cat.-no. 01 00120 70 04 0018)
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 |

Only freeze once! Discard contaminated specimens!

## Assay Procedure

***Application sheets for automated systems are available on request.***

| | |
|---|---|
| Wavelength | 340 nm, Hg 365 nm, Hg 334 nm |
| Optical path | 1 cm |
| Temperature | 37°C |
| Measurement | Against air |

***Substrate Start***

| | |
|---|---|
| **Sample or calibrator** | 100 µL |
| **Reagent 1** | 1000 µL |
| Mix, incubate for 5 min., then add: | |
| **Reagent 2** | 250 µL |
| Mix, read absorbance after 1 min and start stopwatch. | |
| Read absorbance again 1, 2 and 3 min thereafter. | |

***Sample Start***

*Do not use sample start with Pyridoxalphosphate!*

| | |
|---|---|
| **Sample or calibrator** | 100 µL |
| **Mono reagent** | 1000 µL |
| Mix, read absorbance after 1 min. and start stopwatch. | |
| Read absorbance again 1, 2 and 3 min thereafter. | |

## Calculation

**With factor**

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

**ΔA/min x factor = ALAT activity [U/L]**

| | Substrate Start | Sample Start |
|---|---|---|
| 340 nm | 2143 | 1745 |
| 334 nm | 2184 | 1780 |
| 365 nm | 3971 | 3235 |

**With calibrator**

$$\text{ALAT [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Calibrator [U/L]}$$

**Conversion factor**

ALAT [U/L] x 0.0167 = ALAT [µkat/L]

## Calibrators and Controls

For the calibration of automated photometric systems, DiaSystem UniCal CC calibrator is recommended. This method has been standardized against the original IFCC formulation. For internal quality control, Diasystem UniLab N and UniLab P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

| | Cat. No. | Kit size |
|---|---|---|
| UniCal CC | 06 00122 70 04 0018 | 6 x 3 mL |
| UniLab N | 07 00123 70 05 0030 | 6 x 5 mL |
| UniLab P | 07 00124 70 05 0030 | 6 x 5 mL |

## Performance Characteristics

**Measuring range**

On automated systems the test is suitable for the determination of ALAT activities up to 600 U/L. In case of a manual procedure, the test is suitable for ALAT activities which correspond to a maximum of ΔA/min of 0.16 at 340 and 334 nm or 0.08 at 365 nm. If such values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

**Specificity/Interferences**

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 400 mg/dL and lipemia up to 2000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [6].

**Sensitivity/Limit of Detection**

The lower limit of detection is 4 U/L.

**Precision**

***Without Pyridoxal-5-phosphate***

| Intra-assay precision n = 20 | Mean [U/L] | SD [U/L] | CV [%] |
|---|---|---|---|
| Sample 1 | 22.2 | 1.38 | 6.22 |
| Sample 2 | 44.8 | 1.17 | 2.62 |
| Sample 3 | 101 | 1.02 | 1.00 |

| Inter-assay precision n = 20 | Mean [U/L] | SD [U/L] | CV [%] |
|---|---|---|---|
| Sample 1 | 22.8 | 0.70 | 3.08 |
| Sample 2 | 42.6 | 0.68 | 1.60 |
| Sample 3 | 99.3 | 0.92 | 0.92 |

***With Pyridoxal-5-phosphate***

| Intra-assay precision n = 20 | Meany [U/L] | SD [U/L] | CV [%] |
|---|---|---|---|
| Sample 1 | 33.8 | 1.25 | 3.71 |
| Sample 2 | 72.0 | 2.04 | 2.83 |
| Sample 3 | 128 | 2.77 | 2.16 |

| Inter-assay precision n = 20 | Mean U/L] | SD [U/L] | CV [%] |
|---|---|---|---|
| Sample 1 | 33.3 | 0.99 | 2.96 |
| Sample 2 | 72.1 | 1.36 | 1.88 |
| Sample 3 | 133 | 1.76 | 1.32 |

**Method Comparison**

***With Pyridoxal-5-phosphate***

A comparison of DiaSystem ALAT (GPT) FS with P-5-P (y) and the IFCC reference reagent (x) using 51 samples gave following results:
y = 1.000 x – 0.200 U/L; r = 0.999

A comparison of DiaSystem ALAT (GPT) FS with P-5-P (y) and a commercially available test (x) using 51 samples gave following results: y = 0,970 x + 0.531 U/L; r = 1.000

***Without Pyridoxal-5-phosphate***

A comparison of DiaSystem ALT (IFCC) without Pyridoxalphosphate (y) with a commercially available test (x) using 51 samples gave following results:
y =0.971 x + 0.047 U/L; r =1.000

## Reference Range

**With pyridoxal-5-phosphate activation**

| | | | |
|---|---|---|---|
| Women [7] | | < 34 U/L | < 0.57 µkat/L |
| Men [7] | | < 45 U/L | < 0.75 µkat/L |
| Children [1] | 1 – 30 day(s) | < 25 U/L | < 0.42 µkat/L |
| | 2 – 12 months | < 35 U/L | < 0.58 µkat/L |
| | 1 – 3 year(s) | < 30 U/L | < 0.50 µkat/L |
| | 4 – 6 years | < 25 U/L | < 0.42 µkat/L |
| | 7 – 9 years | < 25 U/L | < 0.42 µkat/L |
| | 10 – 18 years | < 30 U/L | < 0.50 µkat/L |

**Without pyridoxal-5-phosphate activation**

| | | |
|---|---|---|
| Women [8,9] | < 31 U/L | < 0.52 µkat/L |
| Men [8,9] | < 41 U/L | < 0.68 µkat/L |

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

## Literature


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