# ALT/GPT Detection Assay

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## Abstract

**Intended use:**

Kinetic system for Alanine Aminotransferase (ALT/ GPT) activity determination.

**Teste Principle:**

**ALT** catalyzes the transfer of amino groups from Alanine to Ketoglutarate, yielding pyruvate and Glutamate. The pyruvate is reduced to lactate by for action of the lactate dehydrogenase (LDH) which oxidizes NADH to $NAD^+$.

The reduction of the absorbance at 340 nm, as consequence of NADH oxidation, must be photometricaly monitored, and is proportional to the ALT activity in the sample.

Aminotransferase determination involves the following reactions:

L-alanine + Ketoglutarate ---(ALT) → Pyruvate + L-Glutamate

Pyruvate + NADH ---(LDH)→ NAD + L-lactate


**Citation:** Zumira Carneiro ALT/GPT Detection Assay. **protocols.io**
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## Guidelines

Reagents are for “in vitro” diagnostic use. Use the reagents according to the working procedures for clinical laboratories.

Storage and stability - Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label.



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## Before start

Spectrophotometer.

- Micropipettes and pipettes for measuring the stated volumes

- Water bath at temperature indicated under PROCEDURE.

- Stopwatch.

## Materials

✓ Reagent 1- TRIS buffer (132.5 mmol/L), L-Alanine (687.5 mmol/L); LDH (≥2300 U/L) and sodium azide (0.095%). by Contributed by users

✓ Reagent 2 - TRIS buffer (20 mmol/L), NADH (1320 µmol/L); Ketoglutarate (82.5 mmol/L) and sodium azide (0.095%). by Contributed by users

✓ Reagent 3 - TRIS buffer (20 mmol/L), pyroxal phosphate (11.1 mmol/L); sodium azide (0.095%). by Contributed by users

## Protocol

Step 1

**Step 1.**

1. In order to achieve traceable results to IFCC[1] Procedure, is needed the use of the two-reagent method, to occur the enzyme total activation by the pyridoxal phosphate.

2. **Preparation the reagent:** Add 0.300 mL of the Reagent 3 to a bottle of Reagent 1(24 mL) and mix. Stability: 21 days at 2 – 8 $^{0}$C and 24 hours 15 –25 $^{0}$C when no chemical or microbial contamination occurs. Optionally, a lower volume of the mixture (Reagent 1 + Reagent 3) may be prepared by using one part of the Reagent 3 to 80 parts of

Reference: 'Reference procedure for the Measurement of catalytic Concentration of Alanine Aminotransferase. Cin Chem Lab Med 2002, 40 (70): 718-24.'

Step 2

**Step 2.**

1. In a test tube labeled “test” or “Calibrator”, Add 0.160 mL of the mixture Reagent 1 + Reagent 3.

Step 3

**Step 3.**



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1. Add 0.020 mL of the sample or enzymes calibrator, homogenize and incubate a water- bath at 37 ± 0.2 $^{0}$C. Wait five minutes. After this incubation it is possible wait until 30 minutes to start the kinetic determination with the addition of the Reagent 2.

Step 4

**Step 4.**

Perform a water Blank measurement at 340 nm.

Step 5

**Step 5.**

1. 0.040 mL of the Reagent 2, homogenize and transfer immediately to a cuvette at 37 ± 0.2 $^{0}$C. Wait one minute.

Step 6

**Step 6.**

1. Measure the initial absorbance ($A_1$). and start simultaneously the timer. Measure the absorbance again after 2 minutes ($A_2$).

CALCULATIONS

**Step 7.**

It is a usual procedure calculated the enzymatic activity results using a theoretical factor achieved in reaction optimum conditions, described below:

Wavelength: 340 nm

Cuvette at 37 ± 0.2 $^{0}$C, 10 mm light path.

Pass band ≤2 nm

Sray light ≤ 0.1%

If one of the correlated parameters is modified, it is recommended to apply an enzymes calibrator indicated by the reagent manufacturer.  Labtest Diagnostica recommends Calibra series to perform the ALT/GPT system calibration.

DA/minute(test or calibrator) = (A1-A2)/2

Factor: (Calibrator activity)/(DA/minute (test)  x factor

ALT activity (U/L) = DA/minute (test) x Factor



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If all the correlated parameters are fulfilled, the theoretical factor (1746) can be applied.

## Warnings

The reagents contain sodium azide as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get prevent azide accumulation.



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