

AST/GOT Detection Assay

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Abstract

Intended use:

Kinetic system for Aspartate Aminotransferase (AST) or Glutamic oxalacetic transaminase (GOT) determination.

Teste Principle:

AST catalyzes specifically the transfer of amino groups of aspartic acid to a Ketoglutarate yielding oxalacetate and Glutamate. The oxalacetate is reduced to malate by the enzyme malate dehydrogenase (MDH) and coenzyme NADH is oxidized NAD.

The reduction of the absorbance at 340 nm, as consequence of NADH oxidation, is determined photometrically and is direct proportional to the AST activity in the sample.

Aminotransferase determination involves the following reactions:

L- Aspartate + Ketoglutarate $\xrightarrow{\text{AST}}$ Oxalacetate + L-Glutamate

Oxalacetate + NADH $\xrightarrow{\text{MDH}}$ Malate + NAD

Citation: Zumira Carneiro AST/GOT Detection Assay. **protocols.io**

[dx.doi.org/10.17504/protocols.io.k92cz8e](https://doi.org/10.17504/protocols.io.k92cz8e)

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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.

Before start

Spectrophotometer.

- Micropipettes and pipettes for measuring the stated volumes

- Water bath at temperature indicated under PROCEDURE.
- Stopwatch.

Materials

- ✓ Reagent 3 - TRIS buffer (20 mmol/L), pyroxal phosphate (11.1 mmol/L); sodium azide (0.095%). by Contributed by users
- ✓ Reagent 1 TRIS buffer (105 mmol/L), L-Aspartate (330 mmol/L); MDH (≥ 825 U/L) and sodium azide (0.095%). by Contributed by users
- ✓ Reagent 2 TRIS buffer (20 mmol/L), NADH (1320 μ mol/L); Ketoglutarate (66 mmol/L) and sodium azide (0.095%). by Contributed by users

Protocol

Step 1

Step 1.

1. In order to achieve traceable results to IFCC¹ 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 $2 - 8^{\circ}\text{C}$ and 24 hours $15 - 25^{\circ}\text{C}$ 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.

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

Step 4

Step 4.

Perform a water Blank measurement at 340 nm.

Step 5

Step 5.

1. 0.400 mL of the Reagent 2, homogenize and transfer immediately to a cuvette at $37 \pm 0.2^{\circ}\text{C}$. 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 $37 \pm 0.2^{\circ}\text{C}$, 10 mm light path.

Pass band ≤ 2 nm

Sray light $\leq 0.1\%$

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 AST/GOT system calibration.

$$\text{DA/minute(test or calibrator)} = (A_1 - A_2)/2$$

$$\text{Factor: (Calibrator activity)/(DA/minute (test) x factor}$$

$$\text{AST activity (U/L)} = \text{DA/minute (test) x Factor}$$

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.