ALT / Alanine Transaminase Assay Kit

ALT / Alanine Transaminas Assay Kit is an assay kit for the quantification of ALT / Alanine Transaminase in serum and plasma.

Catalog number: ARG81298

Package: 100 tests

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INTRODUCTION

Alanine transaminase (ALT) is a transaminase enzyme (EC 2.6.1.2). It is also called alanine aminotransferase (ALAT) and was formerly called serum glutamate-pyruvate transaminase (SGPT) or serum glutamic-pyruvic transaminase (SGPT) and was first characterized in the mid-1950s by Arthur Karmen and colleagues. ALT is found in plasma and in various body tissues but is most common in the liver. It catalyzes the two parts of the alanine cycle. Serum ALT level, serum AST (aspartate transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health. The tests are part of blood panels.

ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate.

L-alanine + α-ketoglutarate ⇌ pyruvate + L-glutamate

ALT is commonly measured clinically as part of liver function tests and is a component of the AST/ALT ratio. When used in diagnostics, it is almost always measured in international units/liter (IU/L) or μkat. While sources vary on specific reference range values for patients, 0-40 IU/L is the standard reference range for experimental studies. [Wikipedia]

PRINCIPLE OF THE ASSAY

ALT is an aminotransferase that catalyzes the reversible transfer of the amino group from L-alanine to α-ketoglutarate. It facilitates the conversion of L-alanine and α-ketoglutarate to pyruvate and glutamate. And then pyruvate and NADH are converted to lactate and NAD+ by lactate dehydrogenase (LDH) and this step is slightly reversible reaction. The assay kit is an indirect enzymatic method to detect the decrease level of NADH by absorbance at 340 nm and it is proportionate to ALT activity. The total incubation time is 10 min only.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	24 ml	-20°C
Enzyme Mixture	120 µl	-20°C
Cosubstrate	600 µl	-20°C
NADH	1 vial (lyophilized)	-20°C

The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt, 3 weeks after reconstitution.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 340 nm
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Heat block or hot water bath or oven
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at -20°C at all times.
- Keep thawed Enzyme Mixture stock on ice before use.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- **NADH Reagent:** Reconstitute NADH with 1000 µl of distilled water, to yield a concentration of 10 mM. Unused reconstituted NADH reagent is stable for three weeks when stored at -20°C.
- **Sample / Standard Working Reagent:** *Prepare before use,* for each well mix the following reagents:

  200 µl Assay Buffer,

  5 µl Cosubstrate,

  1 µl Enzyme Mixture (LDH)

  4 µl of reconstituted NADH reagent

  Warm Working Reagent to assay temperature (e.g. RT or 37°C) before use. Add 200 µl per well in sample and standard wells.
- **Blank Working Reagent:** *Prepare before use,* for each well mix the following reagents:

  200 µl Assay Buffer,

  5 µl Cosubstrate,

  1 µl Enzyme Mixture (LDH)

  4 µl of distilled water.

  Warm Working Reagent to assay temperature (e.g. RT or 37°C) before use. Add 200 µl per well in blank wells.
- **Enzyme Mixture:** Keep thawed enzyme (LDH) on ice before use and return unused Enzyme to ice or -20°C immediately.
- **Assay buffer:** Assay buffer is ready to use, mix it well by vigorous shaking before use.

ASSAY PROCEDURE

Working reagents, microplate and spectrophotometer should be equilibrated to assay temperature before use, the kit could be assayed at room temperature (RT) or 37°C. Standards and samples should be assayed in at least duplicates.

1. Add **20 µl** of **distilled water** in standard well and blank well.
2. Add **20 µl** of **samples** into the corresponding sample wells. Keep plate at the assay temperature. (e.g. RT or 37°C).
3. Prepare Sample / Standard Working Reagent for Sample and Standard and Blank Working Reagent for blank as the instruction in REAGENT PREPARATION section.
4. Add **200 µl** of **Sample / Standard Working Reagent** in the Sample and Standard wells.
5. Add **200 µl** of **Blank Working Reagent** in the Blank wells.
6. Tap the plate to mix it well immediately. Incubation the plate in the **assay temperature (RT or 37°C)**
7. **Read O.D.** with a microplate reader at **340 nm** at **5 min** and at **10 min** after incubate in the assay temperature.

Summary of ALT / Alanine Transaminase Assay Procedure

Reagent	Sample	Standard	Blank
Distilled water	-	20 µl	20 µl
Sample	20 µl	-	-
Keep plate at the assay temperature. (e.g. RT or 37°C).			
Sample/Standard Working Reagent	200 µl	200 µl	-
Blank Working Reagent	-	-	200 µl
Tap the plate to mix it well immediately.			
Incubation the plate in the assay temperature (RT or 37°C)			
Read O.D. with a microplate reader at **340 nm** at **5 min** and at **10 min.**			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. For each Sample and standard, calculate the rate of NADH consumption by subtracting the OD at 10 min from the OD at 5 min (ΔOD). The descriptions are as below.

ΔOD_{Sample} = (OD_{Sample} at 5 min)- (OD_{Sample} at 10 min)

ΔOD_{NADH} = ($OD_{Standard}$ at 5 min)- ($OD_{Standard}$ at 10 min)

$OD_{Standard\text{-}5}$ = $OD_{Standard}$ at 5 min

OD_{Blank} = OD_{Blank} at 5 min

3. Determine ALT activity using the following equation:

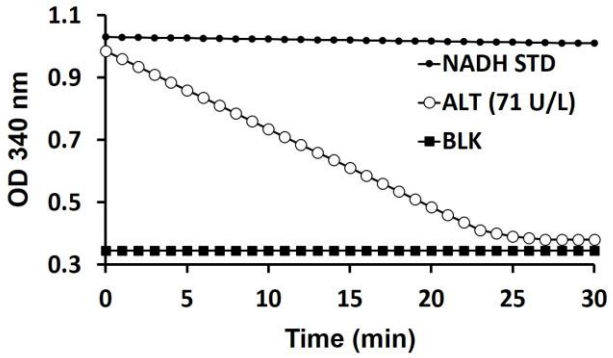
$$ALT\ (U/L) = 381 \times [(\Delta OD_{Sample} - \Delta OD_{NADH}) / (OD_{Standard\text{-}5} - OD_{Blank})]$$

Note: the factor 381 comes from:
10 mM NADH X [4 µl (Vol.NADH)/210 µl (Vol.WorkReagent)]
X [200 µl (Vol.WorkReagent)/220 µl (Vol.Total)] X [11 (sample dilution)/5 min]
= 0.381 mM/min = 381 µM/min

4. If the calculated ALT activity is higher than 100 U/L, dilute sample in Assay Buffer and repeat assay. Multiply results by the dilution factor.

5. Unit definition: 1 Unit (U) of ALT will catalyze the conversion of 1 µmole of alanine to pyruvate per min at pH 7.7.

EXAMPLE OF TYPICAL STANDARD CURVE



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of ALT / Alanine Transaminase ranged from 2- 100 U/l. The mean MDD was 2 U/l