**MANUAL**

# Alanine Transaminase (ALT) Activity Assay Kit (Colorimetric)

Catalog #: MA-ALT

ISO 13485:2016

## Introduction

Alanine transaminase (ALT), also known as alanine aminotransferase (ALAT) or serum glutamic pyruvic transaminase (sGPT), is a homodimeric enzyme dependent on cytoplasmic pyridoxal phosphate. It plays a vital role in cellular nitrogen metabolism, amino acid metabolism, and liver gluconeogenesis. ALT catalyzes the reversible transamination between alanine and α-ketoglutarate to form pyruvate and glutamate. ALT is found mainly in the liver and, to a lesser extent, in kidney, heart, muscle, and pancreas tissues. Normal serum levels of ALT are low, and an elevation in serum ALT activity is a widely used marker for liver damage.

The RayBio® Alanine Transaminase Activity Assay Kit offers a user-friendly, reliable, and highly sensitive method for quantifying ALT activity in plasma, serum, cell lysates, and other biological fluid samples. In this assay, ALT catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. Simultaneously, lactate dehydrogenase catalyzes the reduction of pyruvate and the oxidation of NADH to NAD. The resultant rate of absorbance decrease is directly proportional to ALT activity.

## Storage

The entire kit may be stored at 2–8 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

| Component | Size / Description | Storage After Preparation |
|---|---|---|
| Microplate | A 96-well (12 strips x 8 wells) plate | RT* |
| Sample Buffer | 10 ml | 2–8 °C |
| Assay Buffer | 10 ml | 2–8 °C |
| Enzyme Mix Solution | 2 ml | 2–8 °C |
| Alanine Transaminase (ALT) Positive Control | 1 Vial (10 µl) | 2–8 °C |

*RT = room temperature*

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

## Additional Materials Required

1. Microplate reader capable of measuring absorbance at 340 nm at 37°C
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. Incubator at 37ºC
6. 15 ml conical tubes

## Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, normal human plasma has ALT activity in the range of 8-40 U/L. No dilution/Neat is recommended for a typical plasma sample.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, normal human serum has ALT activity in the range of 8-40 U/L. No dilution/Neat is recommended for a typical serum sample.

*NOTE:*
If the calculated ALT activity of the sample is higher than 500 U/L, dilute the sample in Sample Buffer and repeat the assay.

# Reagent Preparation

## A. Working Solution

Mix Assay Buffer and Enzyme Mix Solution at a volumetric ratio of 5:1 to make the Working Solution. For example, mix 9.5ml of Assay Buffer and 1.9ml of Enzyme Mix Solution to prepare sufficient Working Solution for one 96-well plate. Mix well. Protect from light. The Working Solution is stable for 14 days at 4 ºC.

## B. Positive Control

Alanine Transaminase (ALT) Positive Control: pipette 1 µL of ALT Positive Control into 99 µL Sample Buffer to create a positive control stock, mix well. Then pipette 1 µL of the positive control stock into 99 µL Sample Buffer to prepare the positive control sample. Mix well.

# Assay Procedure

Positive control, samples, and Sample Buffer (used as a blank) should be assayed in duplicate or triplicate. A freshly prepared positive control should be used each time the assay is performed.

1. Set up a microplate reader or a microplate incubator at 37°C.
2. Prepare Working Solution (See Reagent Preparation, section A), and incubate it at 37°C for at least five minutes.
3. Prepare Positive Control (See Reagent Preparation, section B).
4. Pipette 10 µL of sample, Sample Buffer (as the blank) and Positive Control into each well of the 96-well plate.
5. Transfer 100 µL of pre-warmed Working Solution into each well (it is recommended to use a multi-channel pipette), mix and incubate at 37°C for one minute.
6. After one minute, read and record absorbance at 340nm (A1 Reading). Repeat readings every minute for the next four minutes at 37°C. (A2, A3, A4, A5 Reading).

## Calculation of Results

Calculate the mean absorbance for each set of duplicate/triplicate samples, Positive Control, and Sample Buffer blank.

Calculate the average absorbance difference per minute $\Delta OD_{340nm}$/min for each sample, the Positive Control, and Sample Buffer blank.

$$\Delta OD_{340nm}/min = \frac{(A1-A2) + (A2-A3) + (A3-A4) + (A4-A5)}{4}$$

Subtract the absorbance difference per minute of the Blank wells from absorbance difference per minute of the sample and positive control, this is the corrected absorbance difference per minute.

$$\Delta OD'_{340nm}/min = \Delta OD_{340nm}/min - \Delta OD^{Blank}_{340nm}/min$$

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

$$ALT\ activity\ (U/L) = \frac{\Delta OD'_{340nm} \times 110\ \mu l \times 1000}{1.836\ mM^{-1} \times 10\ \mu l} \times sample\ dilution$$

Where: $\Delta OD'_{340nm}$ */min* = Corrected absorbance change per minute

1000 = Conversion of U/ml to U/L

1.836 $mM^{-1}$ is the adjusted extinction coefficient for NADH at 340nm with the current path length of the solution in a 96-well plate.

*ALT activity International System of Units (SI Units) (nkat/L) = ALT activity (U/L)*16.67*

*Note:* If the calculated ALT activity of the sample is higher than 500 U/L, dilute the sample in Sample Buffer and repeat the assay.

## A. Typical Data

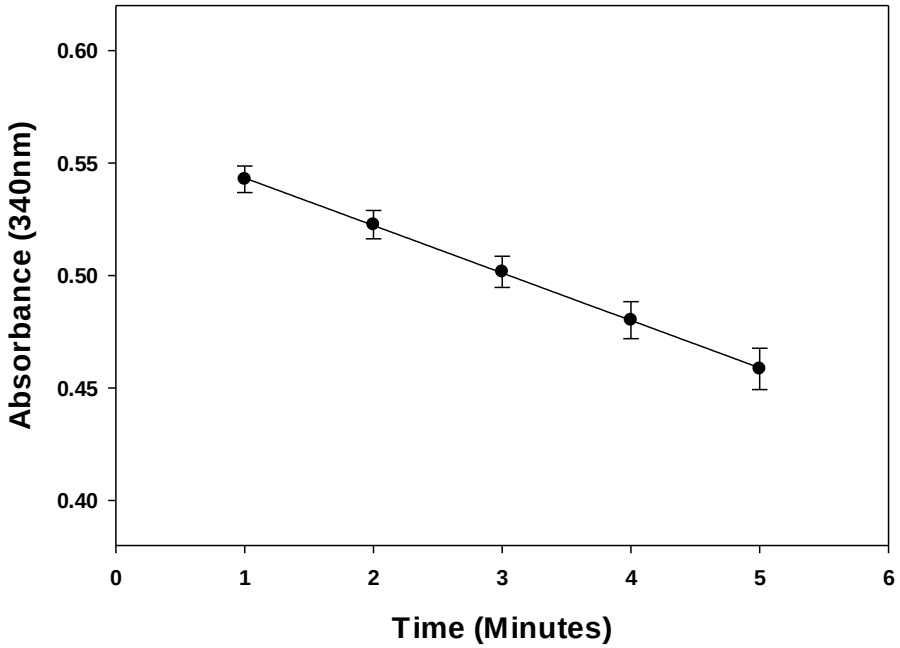


Figure 1. Example of OD value of Alanine Transaminase (ALT) Positive Control verse Time. This example is for demonstration purposes only.

| | ΔOD340nm/min | ALT activity (U/L) |
|---|---|---|
| **Human Serum** | 0.002 | 11.9841 |
| **Human Plasma (EDTA)** | 0.002 | 11.9841 |
| **Human Plasma (Citrate)** | 0.00175 | 10.4860 |
| **Human Plasma (Heparin)** | 0.0015 | 8.9880 |
| **HepG2 cell lysate (protein concentration 4.28mg/ml)** | 0.03083 | 184.7545 |
| **ALT Positive Control** | 0.02858 | 171.2729 |

Table 1. Typical data of different sample types.

## B. Linear Range

Up to 500 U/L ALT activity

## C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 8.5%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 9.3%.

This product is for research use only.