Product Manual

Alanine Aminotransferase (ALT) Activity Assay Kit (Fluorometric)

Catalog Number

MET-5124 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Alanine Aminotransferase (ALT), also known as serum glutamic-pyruvic transaminase (SGPT), is an enzyme that catalyzes the transfer of an amino group from alanine to α-ketoglutarate, thereby producing glutamate and pyruvate (Figure 1). This pyridoxal phosphate dependent transaminase is found primarily in serum and the liver, but it can also be found in various other body tissues. ALT is usually measured as a clinical marker of liver function to ascertain liver health. Hepatocellular injury often leads to an increase in ALT levels, which are usually measured in units per liter (U/L).

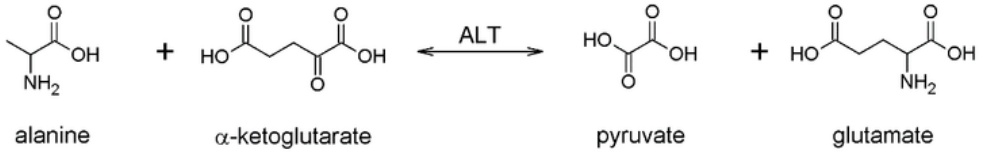

Figure 1: ALT Reaction Principle.

Cell Biolabs' Alanine Aminotransferase (ALT) Assay Kit is a simple fluorometric assay that measures the activity of ALT present within plasma, serum, tissue homogenates, or cell suspensions in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and samples. An ALT positive control is also provided.

Assay Principle

Cell Biolabs' Alanine Aminotransferase (ALT) Assay Kit measures ALT activity through a series of enzyme driven reactions. ALT in samples reacts with alanine to transfer an amino group to another substrate, producing glutamate and pyruvate. Pyruvate is then detected with the fluorometric probe. Samples and standards are incubated for 30-60 minutes and then read with a standard 96-well fluorometric plate reader (Ex. 530-570 nm/Em. 590-600 nm). The ALT activity is directly proportional to the amount of pyruvate generated in the reaction. Sample ALT levels are determined by comparison with the known pyruvate standards.

Related Products

1. MET-5023: Glycogen Assay (Fluorometric)
2. MET-5029: Pyruvate Assay Kit (Fluorometric)
3. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
4. STA-674: Glutamate Assay Kit
5. STA-681: Glucose Assay Kit (Fluorometric)

Kit Components (shipped on blue ice)

1. ALT Enzyme Mix (Part No. 51241C): One 600 µL amber tube
2. ALT Substrate Mix (Part No. 51242C): One 1 mL tube
3. Pyruvate Standard (Part No. 51243C): One 100 µL tube of a 10 mM solution
4. Fluorescence Probe (100X) (Part No. 260003): One 100 µL tube in DMSO
5. HRP (Part No. 234402): One 100 µL tube of a 100 U/mL solution in glycerol
6. ALT Positive Control (Part No. 51244C): One 50 µL tube of 100 U/mL enzyme
7. 10X Assay Buffer (Part No. 50292A): One 25 mL bottle

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Standard 96-well fluorescence microtiter plate

Storage

Upon receipt, store the ALT Substrate Mix and 10X Assay Buffer at 4ºC. Store the remaining components at -20ºC.

Preparation of Reagents

- 1X Assay Buffer: Warm the 10X Assay Buffer to room temperature prior to using. Dilute the Assay Buffer to 1X with deionized water by diluting the 25 mL Buffer with 225 mL deionized water for 250 mL total. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC up to six months.
- ALT Positive Control: Prior to use, dilute the positive control 1:100 in 1X Assay Buffer. Use only what is needed.
- Reaction Reagent: Prepare a Reaction Reagent by diluting the kit components accordingly. ALT Enzyme Mix 1:17, ALT Substrate Mix 1:10, HRP 1:500, and Fluorescence Probe 1:100 in 1X Assay Buffer. See Table 1 below for examples of Reaction Reagent preparation based on the number of assays employed. Mix thoroughly and protect the solution from light. For best results, place the Reaction Reagent on ice and use within 30 minutes of preparation. Do not store the Reaction Reagent solution.

ALT Enzyme Mix (µL)	ALT Substrate Mix (µL)	HRP (µL)	Fluorescence Probe (100X) (µL)	1X Assay Buffer (µL)	**Number of Assays (100 µL/well)**
588	1000	20	100	8,292	**100**
294	500	10	50	4,146	**50**
147	250	5	25	2,073	**25**

Table 1. Preparation of Reaction Reagent

Note: The Fluorescence Probe is light sensitive and must be stored accordingly.

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Tissues: Weigh 500-1000 mg of sample and mince with scissors and a dounce until tissue is thoroughly liquified. Add 2 mL of 1X Assay Buffer or PBS and further sonicate the homogenate for several cycles on ice. Centrifuge 10 minutes at 12,000 x g to remove debris. Recover the supernatant and recentrifuge in a separate tube to clarify it further. Recover supernatant in a fresh microcentrifuge tube and incubate on ice. Prepare samples for testing and store the remaining supernatant at -80ºC. Prepare further dilutions in 1X Assay Buffer.

- Cell Suspensions: Prepare cells at 1x 10^6 cells/mL and rapidly homogenize the cell pellet with 0.2 mL cold PBS or 1X Assay Buffer. Centrifuge 10 minutes at 12,000 x g to remove debris. Recover supernatant in a fresh microcentrifuge tube and incubate on ice. Prepare samples for testing and store the remaining supernatant at -80ºC. Prepare further dilutions in 1X Assay Buffer.
- Serum: Collect blood without using an anticoagulant. Allow blood to clot for 30 minutes at room temperature. Centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the serum layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform serum dilutions in 1X Assay Buffer. Perform several serial dilutions to ensure values are within the range of the standard curve.
- Plasma: Collect blood with heparin or citrate (EDTA could cause a quenching effect) and centrifuge at 1000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform plasma dilutions in 1X Assay Buffer. Perform several serial dilutions to ensure values are within the range of the standard curve.

Notes:

1. *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.*
2. *Avoid samples containing DTT or β-mercaptoethanol since the fluorescence probe is not stable in the presence of thiols (above 10 µM).*
3. *The Fluorescence Probe is unstable at high pH (>8.5).*

Preparation of Pyruvate Standard Curve

Prepare fresh pyruvate standards in the range of 0-40 µM by diluting the provided 10 mM Pyruvate Standard according to Table 2.

Tubes	10 mM Pyruvate Standard (µL)	1X PBS (µL)	Final Pyruvate Concentration (µM)	Pyruvate Quantity (nmoles/well) *
1	4	996	40	2
2	200 of Tube #1	200	20	1
3	200 of Tube #2	200	10	0.5
4	200 of Tube #3	200	5	0.25
5	200 of Tube #4	200	2.5	0.125
6	200 of Tube #5	200	1.25	0.063
7	200 of Tube #6	200	0.625	0.031
8	200 of Tube #7	200	0.313	0.016
9	200 of Tube #8	200	0.156	0.008
10	0	200	0	0

Table 2. Preparation of Pyruvate Standards.

*Note: *Based on 50 µL volume/well. Do not store diluted pyruvate standard solutions.*

Assay Protocol

Each pyruvate standard, ALT positive control and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve and positive control should be used each time the assay is performed.

Note: The assay is continuous, thereby allowing for readings at multiple time points. This may be necessary in order to ensure the values of unknowns fall within the linear range of the standard curve.

1. Add 50 µL of the diluted pyruvate standards, positive control or samples to a 96-well microtiter plate.
2. Add 100 µL of the prepared Reaction Reagent to each well and mix the well contents thoroughly.
3. Immediately read the fluorescence of each microwell used on a fluorometer using 530-570 nm excitation filter and 590-600 nm emission filter. The initial time point is ($T_{Initial}$) and the initial plate fluorescence reading is ($F_{initial}$). Cover plate to protect from light and continue to incubate at 37ºC for up to 30-60 minutes.

Note: If measuring multiple time points, begin reading samples after adding the Reaction Reagent at every set time point (e.g., every 5 minutes). An initial lag phase (~2-5 minutes) may precede color development. After this lag phase and color begins to develop, take the initial measurement ($F_{Initial}$). Continue taking measurements until the reaction is complete, which is indicated by one of the following:

- *The fluorescent signal of the most active sample exceeds the high end of the linear range of the standard curve. The penultimate (immediately prior) reading, where the RFU value still falls within the linear range of the curve, is used to determine ALT activity. This penultimate reading is (F_{Final}) at (T_{Final}) time point. The RFU values for the initial and final measurements must fall within the linear range of the standard to be accurate.*
- *The fluorescent signal of the most active sample does not significantly change from the prior reading. This indicates the reaction has reached a plateau and is not likely to continue.*

4. Once the assay is complete, read the fluorescence of each microwell on a fluorometer using 530-570 nm excitation filter and 590-600 nm emission filter. This is the final time point (T_{Final}) plate reading (F_{final}).

Example of Results

The following figures demonstrate typical ALT Activity Assay results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

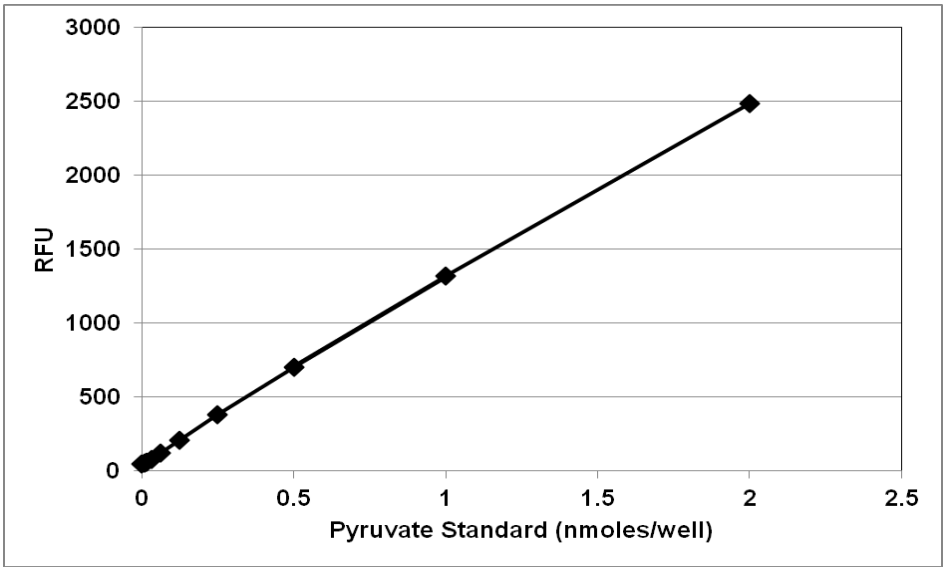

Figure 2: Example Pyruvate Standard Curve.

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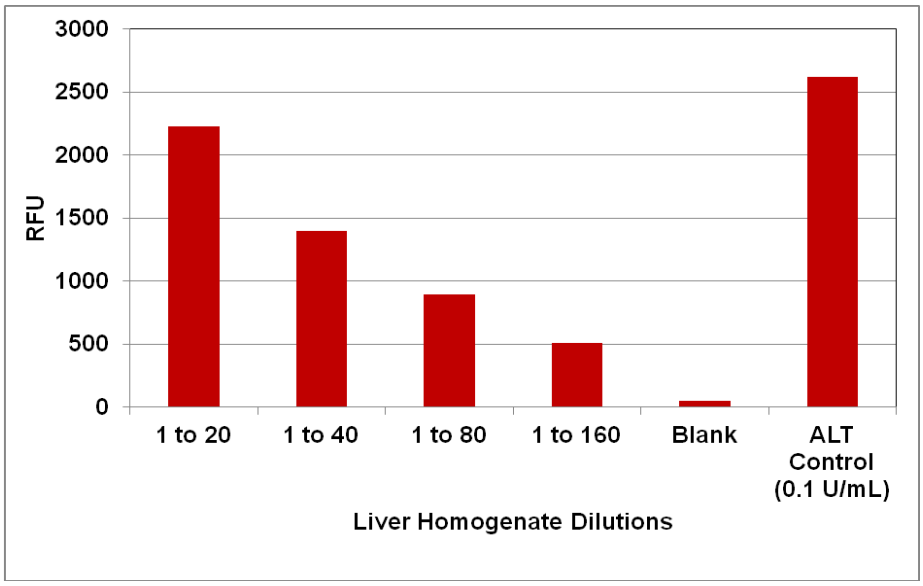

Figure 3: Liver Homogenates. Chicken livers were homogenized and sonicated in cold 1X Assay Buffer. Upon centrifugation, the homogenate was tested according to the assay protocol.

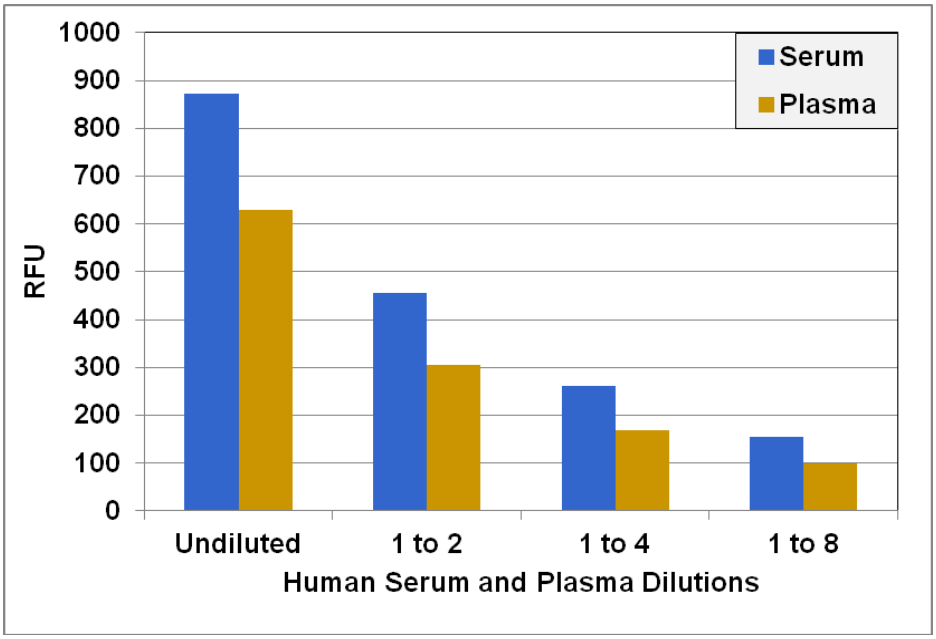

Figure 4: Human Serum and Plasma. Human plasma and serum were both diluted in 1X Assay Buffer and tested according to the assay protocol.

Calculation of Results

1. Determine the average fluorescence values for every sample, control, and standard. Subtract the average zero standard value from itself and all standard and sample values. This is the background corrected fluorescence. Use the T_{Final} readings to plot the pyruvate standard curve graph.
2. Graph the standard curve with the corrected fluorescence values (see Figure 2 for an example standard curve).

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3. Calculate the change in sample fluorescence values (ΔF) between the initial fluorescence $(F_{Initial})$ and the final fluorescence (F_{Final}):

$$\mathbf{(\Delta F) = (F_{Final}) - (F_{Initial})}$$

4. Compare the change in fluorescence (ΔF) of each sample to the pyruvate standard curve to determine the amount of pyruvate produced within the assay. Only use values within the linear range of the standard curve.
5. Determine the ALT activity in milliunits/mL (mU/mL) of a sample using the equation:

   Q = Quantity (in nmoles/well) of pyruvate produced as determined from standard graph
   T = Reaction time (in minutes) determined by $T_{Final} - T_{Initial}$

$$\textbf{ALT Activity (mU/mL)} = \left[\frac{\mathbf{Q}}{\mathbf{(T_{final} - T_{initial}) \times 0.050\ mL^*}} \right]$$

**Note: 50 µL sample volume. Be sure to account for any dilution factors made on unknown samples prior to the assay.*

ALT activity is quantified as nmole/min/mL = milliunit/mL (mU/mL), where 1 milliunit of ALT is the amount of enzyme that generates 1.0 nmole of pyruvate per minute at 37ºC.

References

1. Ishiguro, M., et al. (1991) *Biochemistry* **30**: 10451-10457.
2. Liu Z., et al. (2014) *Int. J. Med. Sci.* **11(9)**: 925-935.
3. Tarao, K., et al. (1999) *Cancer* **86**: 589-595.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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