Technical Bulletin

ALT Activity Assay Kit

Catalogue Number MAK571

Product Description

Alanine Aminotransferase (ALT), also known as serum glutamic-pyruvic transaminase (SGPT), is a pyridoxal-phosphate-dependent enzyme that catalyzes the reversible transfer of an amino group from L-alanine to α-ketoglutarate, generating pyruvate and glutamate. ALT is found primarily in liver and serum but occurs in other tissues as well.

Hepatocellular injury often results in an increase of serum ALT levels and serum ALT levels can be used as a biomarker for liver injury.

The ALT Activity Assay Kit provides a simple and direct procedure for measuring ALT activity in a variety of biological samples.

ALT activity is determined by a coupled enzyme assay, which results in a colorimetric (A570 nm) / fluorometric (λ ex = 535 / λ em = 587 nm) product, proportional to the pyruvate generated.

Unit Definition - One unit of ALT is defined as the amount of enzyme that generates 1.0 µmole of pyruvate per minute at 37 °C

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- ALT Assay Buffer — 25 mL
  Catalogue number MAK571A
- HRP Substrate — 0.2 mL
  Catalogue number MAK571B
- ALT Developer Mix A — 1 Vial
  Catalogue number MAK571C
- ALT Developer Mix B — 1 Vial
  Catalogue number MAK571D
- ALT Substrate A — 1 Vial
  Catalogue number MAK571E
- ALT Substrate B — 1 Vial
  Catalogue number MAK571F
- Pyruvate Standard, 100 mM — 0.1 mL
  Catalogue number MAK571G
- ALT Positive Control — 1 Vial
  Catalogue number MAK571H

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate.
    - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
    - Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent)
    - Cell culture or tissue culture treated plates are not recommended.
- Plate reader that is able to read absorbance and/or fluorescence.
- Pipettors and Pipettes
- Vortex Mixer

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped on dry ice. Store at -20° C upon receipt, protected from light.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

ALT Assay Buffer (MAK571A)

Allow buffer to come to room temperature before use.

HRP Substrate (MAK571B)

Allow reagent to come to room temperature before use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at -20°C.

ALT Developer Mix A (MAK571C)

Reconstitute in 220 µL of ultrapure water. Mix well by pipetting, then aliquot and store at -20°C. Use within two months of reconstitution. Keep on ice while in use. Avoid repeated freeze/thaw cycles.

ALT Developer Mix B (MAK571D)

Reconstitute in 220 µL of ultrapure water. Mix well by pipetting, then aliquot and store at –20°C. Use within two months of reconstitution. Keep on ice while in use. Avoid repeated freeze/thaw cycles.

ALT Substrate A & B (MAK571E & MAK571F)

Reconstitute ALT Substrate A with 1.1 mL of ALT Assay Buffer. Then use all of the Substrate A solution to reconstitute ALT Substrate B. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use. Use within two months of reconstitution. Avoid repeated freeze/thaw cycles.

Pyruvate Standard, 100 mM (MAK571G)

Allow solution to come to room temperature before use. The Pyruvate Standard is 100X concentrated.

ALT Positive Control (MAK571H)

The vial contains approximately 5 µL of ALT enzyme in ammonium sulfate suspension. Dilute in 1 mL of ALT Assay Buffer. Mix well by pipetting, then aliquot and store at –20°C. Keep on ice while in use. Use within two months of reconstitution. Avoid repeated freeze/thaw cycles. Following dilution, the ALT Positive Control is approximately 50X concentrated.

Further dilute by a factor of 50 (20 µL positive control +980 µL ALT Assay Buffer). Then, add 2-10 µL of the ALT Positive Control to wells. Adjust well volume to 20 µL with the ALT Assay Buffer.

Procedure

All samples and standards should be run in triplicates. A convenient customer calculator can be found on the product webpage.

Before beginning, it is advisable to pre-heat the plate reader to 37°C and setup the appropriate reading protocol. The protocol should be as follows:

- Preheat to 37°C.
- Start a 60-minutes kinetics program with 5-minute intervals.
- Each kinetic step includes a 10 second horizontal shake followed by absorbance reading at 570 nm or florescence at λ_{ex} = 535 nm and λ_{em} = 587 nm.

Pyruvate Standards for Colorimetric Detection

1. Dilute 10 µL of the 100 nmole/µL (100 mM) Pyruvate Standard with 990 µl of ALT Assay Buffer to prepare a 1 nmole/µL (1 mM) standard solution.
2. Add 0, 2, 4, 6, 8, and 10 µL of the 1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards.
3. Add ALT Assay Buffer to each well to bring the final volume to 20 µL. See "Table A" in the Customer Calculator.

Pyruvate Standards for Fluorometric Detection

1. Prepare a 1 nmole/µL (1 mM) standard solution from Step 1 of the colorimetric detection standard preparation.
2. Further dilute 10 µL of the 1 nmole/µL standard solution with 90 µL of ALT Assay Buffer to make a 0.1 nmole/µL (0.1 mM) standard solution.
3. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards.
4. Add ALT Assay Buffer to each well to bring the final volume to 20 µL.

Sample Preparation

Both the colorimetric and fluorometric assays require 20 µL of sample for each reaction (well).

Tissue samples or cells should be lysed with a lysis buffer before being assayed. The ALT Assay Buffer does not contain a detergent or lysing agent. Serum samples can be directly added to wells. Add 1–20 µL samples into wells of a 96-well plate.

Note: For unknown samples, it is suggested to test several different sample dilutions to ensure that the readings are within the linear range of the standard curve (such as undiluted, diluted by a factor of 10, factor of 100, and so on).

Note: For samples exhibiting significant background, include a "Sample Blank" for each sample by omitting the ALT Substrates. The Sample Blank readings can then be subtracted from the sample readings. See Table 1.

Note: It is extremely important that no air bubbles are formed in any well. Pipette gently to avoid and blow air lightly to burst any bubbles. Air bubbles will yield significantly erratic readings and adverse results.

Assay Reaction

1. Set up the Reaction Mixes according to Table 1 or Table B if using in the Customer Calculator. 100 µL of the Reaction Mix is required for each reaction well.

Prepare enough Master Reaction Mix for the number of sample wells, positive controls, and standard wells to be performed.

Briefly, multiply the number of wells by 84 to obtain the ALT Assay Buffer volume (µL), multiply by 2 to obtain the HRP Substrate/ ALT Enzyme Mix A / ALT Enzyme Mix B

volume (µL) and multiply by 10 to obtain the ALT Substrate A&B volume (µL).

All samples and standards should be run in technical triplicates. It is highly advisable to use a multi-channel pipettor and troughs, when possible, to minimize the technical error.

The volume in each well before the addition of the mix is 20 µL, for standard wells, positive control wells and sample wells.

Table 1.

Master Reaction Mix

Component	Standards and Samples	Sample Blank
ALT Assay Buffer	84 µL	94 µL
HRP Substrate	2 µL	2 µL
ALT Substrate	10 µL	---------
ALT Enzyme Mix A	2 µL	2 µL
ALT Enzyme Mix B	2 µL	2 µL

2. Add 100 µL of the Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. The volume in each well is now 120 µL, for both standard wells and sample/positive control wells.
3. Insert the plate to the pre-heated (37 °C) plate reader. Take the initial measurement (T initial).

For colorimetric assays, measure the absorbance at 570 nm (A_{570}).

For fluorometric assays, measure fluorescence intensity (FLU initial,λ ex = 535/ λ em = 587 nm).

4. Continue to incubate the plate at 37°C, taking measurements every 5 minutes. Protect the plate from light during the incubation.
5. Continue taking measurements until the value of the most active sample / Positive Control is greater than the value of the highest standard.

At this time, the most active sample/Positive Control is near or exceeds the end of the linear range of the standard curve.

6. The final measurement for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve (see previous step). The time of the penultimate reading is T final.

Note: It is essential the initial and final measurements fall within the linear range of the reaction.

Results

Calculations

Calculate the change in measurement from T initial to T final for the samples and positive control.

$$\Delta A_{570} = (\Delta A_{570})final - (\Delta A_{570})initial$$

Or

$$\Delta FLU = (FLU)final - (FLU)initial$$

Correct for the background by subtracting the value obtained for the 0 (blank) standard from all standard readings.

Plot the pyruvate standard curve using the T final readings. Use the values obtained from the appropriate standard wells to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Compare the Δmeasurement value (ΔA570 or ΔFLU) of each sample to the standard curve to determine the amount of pyruvate generated between T initial and T final (B).

Use the Customer Calculator excel file available at MAK571's webpage.

ALT Activity

The ALT activity of a sample may be determined by the following equation:

$$ALT\ Activity = \frac{B\ x\ Sample\ dilution\ Factor}{\left(T_{final} - T_{initial}\right) x\ V}$$

Where:

B = Amount (nmole) of pyruvate generated in unknown sample well, between T initial and T final, calculated using the standard curve.

Reaction Time = T final – T initial (minutes).
V = sample volume (mL) added to well.
ALT activity is reported as nmole/min/mL = milliunit/mL
Where one milliunit (mU) of ALT is defined as the amount of enzyme that generates 1.0 nmole of pyruvate per minute at 37 °C

For additional assistance, please use the calculator available for the MAK571 ALT Activity Assay Kit on the product webpage.

Figure 1.

Exemplary Colorimetric Standard Curve

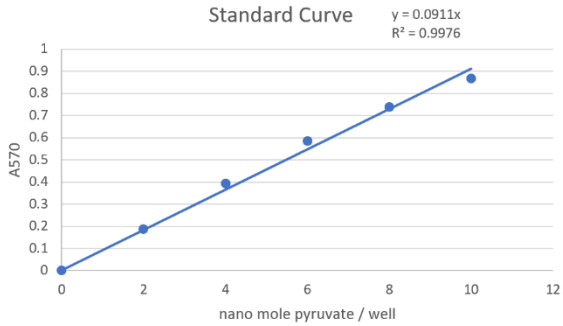
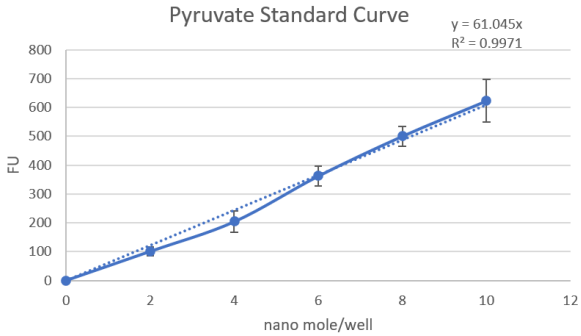


Figure 2.

Exemplary Fluorometric Standard Curve



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Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader reads incorrect wavelength	Check settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates. For colorimetric assays, use clear plates.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of aged kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh reaction mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the reaction mix	Use calibrated pipettes with compatible pipette tips
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin or use web calculator
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

Notice

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