3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Alanine Aminotransferase Activity Assay Kit

Catalog Number **MAK052**
Storage Temperature –20 °C

TECHNICAL BULLETIN

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 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 marker 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 (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the pyruvate generated. 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 assays in 96 well plates.

ALT Assay Buffer Catalog Number MAK052A	25 mL
Fluorescent Peroxidase Substrate, in DMSO Catalog Number MAK052B	0.2 mL
ALT Enzyme Mix Catalog Number MAK052C	1 vl
ALT Substrate Catalog Number MAK052D	1 vl
Pyruvate Standard, 100 nmole/μL Catalog Number MAK052E	0.1 mL
ALT Positive Control Catalog Number MAK052F	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is 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.

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 – Allow buffer to come to room temperature before use.

Fluorescent Peroxidase Substrate – 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 Enzyme Mix – Reconstitute in 220 μL of water. Mix well by pipetting, then aliquot and store at –20 °C. Use within two months of reconstitution.

ALT Substrate – Reconstitute in 1.1 mL of ALT Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use. Use within two months of reconstitution.

ALT Positive Control – Reconstitute in 100 μL of water. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use. Use within two months of reconstitution.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Pyruvate Standards for Colorimetric Detection
Dilute 10 µL of the 100 nmole/µL Pyruvate Standard with 990 µl of ALT Assay Buffer to prepare a 1 nmole/µL standard solution. 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. Add ALT Assay Buffer to each well to bring the volume to 20 µL.

Pyruvate Standards for Fluorometric Detection
Prepare a 1 nmole/µL standard solution as for the colorimetric assay. Dilute 10 µL of the 1 nmole/µL standard solution with 90 µL of ALT Assay Buffer to make a 0.1 nmole/µL standard solution. 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. Add ALT Assay Buffer to each well to bring the volume to 20 µL.

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

Tissue (50 mg) or cells (1×10^6) should be rapidly homogenized with 200 µL of ALT Assay Buffer. Centrifuge at 15,000 × *g* for 10 minutes to remove insoluble materials.

Serum samples can be directly added to wells. Add 1–20 µL samples into wells of a 96 well plate.

Bring samples to a final volume of 20 µL with ALT Assay Buffer. For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

For the positive control (optional), add 5 µL of the ALT Positive Control to wells. Adjust well volume to 20 µL with ALT Assay Buffer.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 100 µL of the Master Reaction Mix is required for each reaction (well). Prepare enough Master Reaction Mix for the number of samples, positive controls, and standards to be performed.

Table 1.
Master Reaction Mix

Reagent	Volume
ALT Assay Buffer	86 µL
Fluorescent Peroxidase Substrate	2 µL
ALT Enzyme Mix	2 µL
ALT Substrate	10 µL

2. Add 100 µL of the Master Reaction Mix to each of the standard, positive control, and test wells. Mix well using a horizontal shaker or by pipetting.

3. After 2–3 minutes, take the initial measurement ($T_{initial}$). For colorimetric assays, measure the absorbance at 570 nm $(A_{570})_1$. For fluorometric assays, measure fluorescence intensity ($FLU_{initial}$, λ_{ex} = 535/λ_{em} = 587 nm).

4. 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 is greater than the value of the highest standard. At this time the most active sample 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. The time of the penultimate reading is T_{final}.

7. Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples and positive control.

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

or

$$\Delta FLU = (FLU_{final}) - (FLU_{initial})$$

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

Results

Calculations

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.

Compare the ∆measurement value (ΔA_{570} or ∆FLU) of each sample to the standard curve to determine the amount of pyruvate generated between $T_{initial}$ and T_{final} (B).

Note: A new standard curve must be set up each time the assay is run.

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

$$\text{ALT Activity} = \frac{B \times \text{Sample Dilution Factor}}{(T_{final} - T_{initial}) \times V}$$

B = Amount (nmole) of pyruvate generated between $T_{initial}$ and T_{final}

$T_{initial}$ = Time of first reading in minutes.

T_{final} = Time of penultimate reading in minutes.

V = sample volume (mL) added to well.

ALT activity 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.

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 at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. 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 samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	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 expired 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 mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	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	Prepare a master Reaction Mix whenever possible
	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
	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

KVG,LS,MAM 05/17-1