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 Assay Kit

Catalog Number **MAK001**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Alanine is a non-essential amino acid that plays a key role in the glucose-alanine cycle between muscle tissue and the liver. In amino acid-degrading tissues such as muscle, amino groups are pooled as glutamate by transamination reactions. The amino group of glutamate is transferred to pyruvate via alanine aminotransferase, forming alanine and α-ketoglutarate. The alanine is passed into the blood and transported to the liver. This reaction is reversed in the liver where pyruvate can be used in gluconeogenesis to form glucose, which may return to other tissues through the circulatory system. Increased alanine levels correlate with higher blood pressure, energy intake, cholesterol levels, and body mass index.

This kit is suitable for alanine detection in cell and tissue culture supernatants, urine, plasma, serum, and other biological samples. Alanine concentration 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 alanine present. Typical detection ranges for this kit are 2–10 nmole (colorimetric) and 0.2–1 nmole (fluorometric).

Components

The kit is sufficient for 100 assays in 96 well plates.

Alanine Assay Buffer Catalog Number MAK001A	25 mL
Alanine Probe, in DMSO Catalog Number MAK001B	0.2 mL
Alanine Converting Enzyme Catalog Number MAK001C	1 vl
Alanine Development Mix Catalog Number MAK001D	1 vl
Alanine Standard, 10 µmole Catalog Number MAK001E	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
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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.

Alanine Assay Buffer – Allow buffer to come to room temperature before use.

Alanine Probe – Warm to room temperature to melt frozen solution prior to use. Store protected from light and moisture at –20 °C. Upon thawing, the Alanine Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the colorimetric Alanine Probe Solution 5 to 10-fold with Alanine Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Alanine Converting Enzyme – Reconstitute in 220 µL of Alanine Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use and protect from light. Use within 2 months of reconstitution.

Alanine Development Mix – Reconstitute in 220 µL of Alanine Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Alanine Standard – Reconstitute in 100 µL of water to generate a 100 mM (100 nmole/µL) Alanine Standard Solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use.

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.

Alanine Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Alanine Standard Solution with 990 µL of water to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, 10 µL of the 1 mM alanine standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Alanine Assay Buffer to each well to bring the volume to 50 µL.

Alanine Standards Fluorometric Detection
Prepare a 1 mM standard solution as for the colorimetric assay. Take 100 µL of the 1 mM alanine standard solution and add to 900 µL of water to make a 0.1 mM alanine standard solution. Add 0, 2, 4, 6, 8, 10 µL of the 0.1 mM alanine 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 Alanine Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Serum samples should be deproteinized before use in assay with a 10 kDa MWCO spin filter. 10–50 µL of deproteinized serum samples can be directly diluted to a final volume of 50 µL with the Alanine Assay Buffer.

Tissue or cells (1×10^6) can be homogenized in 100 µL of the Alanine Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Bring samples to a final volume of 50 µL with Alanine Assay Buffer.
Notes: Samples other than serum may also be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Include a blank sample for each sample by omitting the Alanine Converting Enzyme in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Blank Sample	Samples and Standards
Alanine Assay Buffer	46 µL	44 µL
Alanine Converting Enzyme	–	2 µL
Alanine Development Mix	2 µL	2 µL
Alanine Probe	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the blank, standard, and test wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 37 °C. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) alanine standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate alanine standards to plot a standard curve.

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

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of alanine present in the sample may be determined from the standard curve.

Concentration of Alanine

$$S_a/S_v = C$$

S_a = Amount of alanine in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of alanine in sample

L-Alanine molecular weight: 89.09 g/mole.

Sample Calculation

Amount of alanine (S_a) = 5.84 nmole

Sample volume (S_v) = 50 μL

Concentration of alanine in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 89.09 \text{ ng/nmole} = 10.4 \text{ ng}/\mu\text{L}$$

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
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	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 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 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