ab83394

L-Alanine Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of L-Alanine levels in various samples

This product is for research use only and is not intended for diagnostic use.

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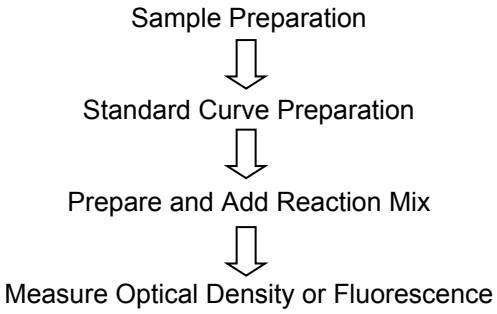
1. Overview

Alanine is the 2nd most abundant of the 20 proteinogenic amino acids. Nonessential, being available from dietary sources, it plays a key role in the glucose-alanine cycle between tissues and liver. In muscle and other tissues that degrade amino acids, amino groups are pooled as glutamate by transamination. Glutamate then transfers the amino group to pyruvate via alanine aminotransferase, forming alanine and α-ketoglutarate. The alanine is passed into the blood and transported to the liver. A reverse of the alanine aminotransferase reaction takes place in liver. Pyruvate can be used in gluconeogenesis, to form glucose which may return to other tissues through the circulatory system. There appears to be a correlation between alanine levels and and higher blood pressure, energy intake, cholesterol levels, and body mass index.

Abcam's L-Alanine Assay Kit provides a sensitive detection method of L-alanine. In the kit, alanine is converted to pyruvate which is specifically detected leading to proportional color (λ=570nm:0-10 nmol) or fluorescence (Ex/Em 535/587nm: 0-1 nmol) development. Serum concentration: ~24-76 µg/mL (~3-9 nmol/10 µL).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Alanine Assay Buffer	25 mL
OxiRed™ Probe	0.2 mL
Converter Enzyme XIV	1 vial
Developer Mix A	1 vial
Alanine Standard	1 vial

PLEASE NOTE: OxiRed™ Probe was previously labelled as OxiRed Probe and Alanine Probe (DMSO), and Developer Mix A as Development Enzyme Mix I and Alanine Development Mix (Lyophilized). The composition has not changed.

* Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

OxiRed™ Probe: Ready to use as supplied. Allow to warm to room temperature to thaw the DMSO solution before use. Store at -20°C, protect from light and moisture. Use within two months.

Converter Enzyme XIV, Developer Mix A: Dissolve separately with 220 µl Assay Buffer. Pipette up and down to dissolve. Aliquot into portions and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep the Alanine Enzyme Mix on ice during the assay and protect from light.

ALANINE STANDARD: Dissolve in 100 µl dH_2O to generate 100 mM (100 nmol/µl) Alanine Standard solution. Keep cold while in use. Store at -20°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Tissues or cells (1×10^6) can be homogenized in 100 µl Assay Buffer centrifuge to remove insoluble material at 13,000 g, 10 minutes.

10-50 µl deproteinized serum and plasma samples can be directly diluted in the Assay Buffer. Bring sample wells to 50 µl/well with Assay Buffer in a 96-well plate.

For unknown samples, we suggest testing several doses to make sure the readings are within the standard curve linear range.

We recommend (per well):

5-50 µl of cell culture medium
Lysates from $2.5x10^5$ cells
0.02-0.1 mg extracted protein from tissue

2. Standard Curve Preparation:

a. For the colorimetric assay:

Dilute 10 µl of the 100mM Alanine standard with 990 µl DI H_2O to generate 1 mM standard Alanine. Add 0, 2, 4, 6, 8, 10 µl of the diluted Alanine standard into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Bring the volume to 50 µl with Assay Buffer.

b. For the fluorometric assay:

Dilute the L-Alanine Standard solution to 0.1 nmol/ µl by adding 10 µl of the L-Alanine Standard to 990 µl of L-Alanine Assay Buffer and mix well. Then take 20 µl into 180 µl of L-Alanine Assay Buffer and mix well.

Add 0, 2, 4, 6, 8, 10 µl into a series of wells of a 96 well plate. Adjust volume to 50 µl/well with L-Alanine Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the L-Alanine Standard.

3. Reaction Mix: Mix enough reagent for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing the following components:

	Alanine Measurement	**Bkgd Control***
Assay Buffer	44 µl	46 µl
Converter Enzyme XIV	2 µl	---
Developer Mix A	2 µl	2 µl
OxiRed™ Probe**	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing Alanine standard, test and background control samples. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.

***Note:** Use background control if high levels of pyruvate are suspected to be in the samples.

****Note:** For the fluorescent assay dilute the OxiRed™ Probe 5-10X to reduce background.

4. Measure OD at 570 nm in a microplate reader or fluorescence using Ex/Em 535/587 nm.

5. Data Analysis

Correct background by subtracting the value derived from the zero Alanine control from all sample readings. The background reading can be significant and must be subtracted from sample readings.

Plot the Alanine standard Curve.

Alanine concentrations of the test samples can then be calculated:

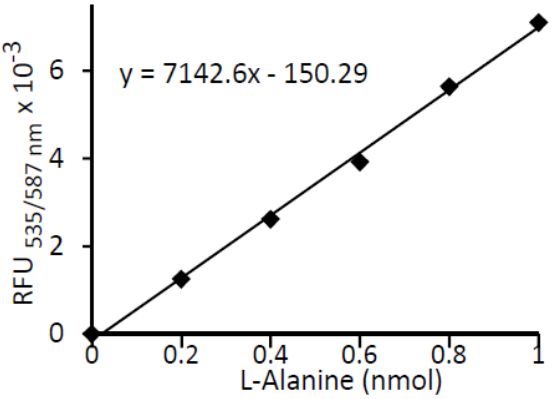
Concentration = Sa / Sv (nmol/µl or mM)

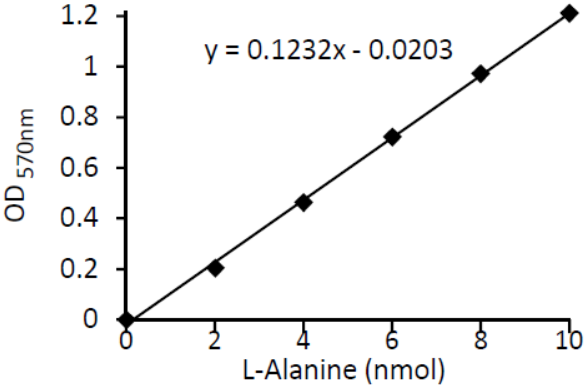
Where:

Sa is the sample amount of unknown (in nmol) from standard curve,

Sv is sample volume (µl) added into the wells.

L-Alanine Molecular Weight is 89.1 g/mol.


y = 7142.6x - 150.29
RFU 535/587 nm x 10-3
0
2
4
6
0
0.2
0.4
0.6
0.8
1
L-Alanine (nmol)


y = 0.1232x - 0.0203
OD 570nm
0
0.2
0.4
0.6
0.8
1
1.2
0
2
4
6
8
10
L-Alanine (nmol)

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)