Technical Bulletin

Alanine Assay Kit

Catalogue number MAK500

Product Description

Alanine is a nonessential amino acid utilized in the glucose-alanine cycle between tissues and the liver. In tissues that metabolize amino acids, amino groups are collected as glutamate by transamination. The amine group is then transferred by alanine transaminase (ALT) from glutamate to pyruvate to form alanine and α-ketoglutarate. The alanine generated is transported to the liver where a reverse ALT reaction occurs and pyruvate is regenerated. Pyruvate is converted through gluconeogenesis to glucose which can then be recirculated to the tissues.

Simple, direct and automation-ready procedures for measuring L-alanine concentration. The Alanine Assay Kit is based on the conversion of alanine into pyruvate, which is then measured directly. The color intensity of the reaction product at 570 nm or fluorescence intensity at λ_{em} = 585 nm/λ_{ex} = 530 nm is directly proportional to the alanine concentration in the sample.

The linear detection range is 1-200 µM for colorimetric assays and 0.4 to 20 µM for fluorometric assays. The kit is suitable for L-alanine determination in serum, plasma, and other biological samples, as well as for studying the effects of drugs on alanine metabolism.

Components

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

- Developer 6 mL
  Catalogue Number MAK500A
- ALT Enzyme 120 µL
  Catalogue Number MAK500B
- Dye Reagent 120 µL
  Catalogue Number MAK500C
- Cosubstrate 600 µL
  Catalogue Number MAK500D
- Alanine Standard (20 mM) 400 µL
  Catalogue Number MAK500E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- Phosphate Buffered Saline (Catalogue Number PPB006 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Assays can be performed at 37 °C or at room temperature. Prior to assay, bring the working reagents, microplate, and spectrophotometer to the desired temperature.

Sample Preparation

Tissue or cell Samples (2×10^6) can be homogenized in 100 µL phosphate buffered saline (pH 7.4). Centrifuge at 14,000 rpm for 5 minutes. Use clear supernatant for assay.

Serum should either be diluted at least 10-fold in purified water or deproteinated using a 10 kDa spin filter (Catalogue UFC8010 or equivalent).

Note: Avoid media with high pyruvate concentrations (for example, DMEM, L-15, F12, etc.).

Add 50 µL of Sample into separate wells of 96-well plate.

Colorimetric Standard Curve Preparation

1. Prepare a 200 µM Alanine Standard by mixing 5 µL of the 20 mM Standard with 495 µL of purified water.
2. Prepare standards in 1.5 mL centrifuge tubes with purified water according to Table 1.

Table 1.

Preparation of Alanine Colorimetric Standards

Well No.	200 µM Standard	Purified Water	Alanine (µM)
1	200 µL	0 µL	200
2	120 µL	80 µL	120
3	60 µL	140 µL	60
4	0 µL	200 µL	0

3. Mix well and transfer 50 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare standards according to Colorimetric Standard Curve Preparation section.
2. Mix 10 µL of the Standards from Colorimetric Procedure with 90 µL of purified according to Table 2.

Table 2.

Preparation of Fluorometric Maltose Standards

Well	Colorimetric Standard	Purified Water	Alanine (µM)
1	10 µL of 200 µM Std	90 µL	20
2	10 µL of 120 µM Std	90 µL	12
3	10 µL of 60 µM Std	90 µL	6
4	-	100 µL	0

3. Mix well and transfer 50 µL of each Standard into separate wells of a black 96 well plate.

Working Reagents

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 3.

Table 3.

Preparation of Working Reagents

Reagent	Working Reagent
Developer	50 µL
ALT Enzyme	1 µL
Cosubstrate	5 µL
Dye Reagent	1 µL

2. Add 50 µL of Working Reagent to each Sample and Alanine Standard well. Tap plate to mix.

Measurement

1. Incubate protected from light for 30 minutes at 37 °C or 60 minutes at RT.
2. Measure the optical density (OD) at 570 nm for colorimetric assay or measure fluorescence at λ_{em} = 585 nm/λ_{ex} = 530 nm

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against the Standard concentrations.
3. Determine the slope and calculate the alanine concentration of Samples using the below equation:

$$\text{Alanine } (\mu M) = \left(\frac{R_{Sample}-R_{Blank}}{\text{Slope}(\mu M^{-1})} \times DF\right)$$

Where:

R_{Sample} = Fluorescence intensity (F) or OD reading of Sample

R_{Blank} = Fluorescence intensity (F) or OD reading of Sample Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Unit conversions: 1 mg/dL alanine equals 112.2 µM, 0.001% or 10 ppm.

Note: If the calculated alanine concentration is higher than 200 µM for the colorimetric assay or higher than 20 µM for the fluorometric assay, dilute Sample in purified water and repeat assay. Multiply result by the dilution factor (DF).

Figure 1.

Typical Colorimetric Alanine Standard Curve

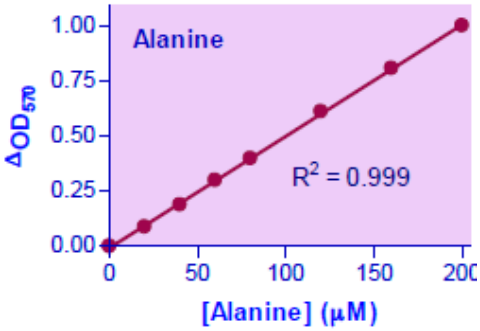
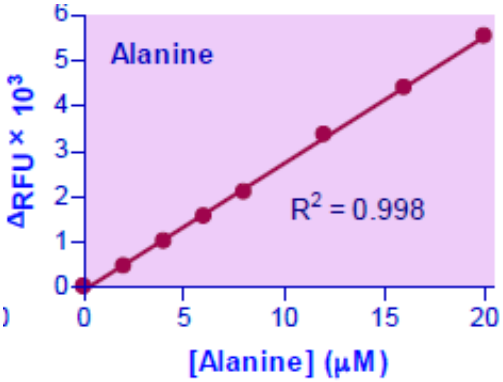


Figure 2.

Typical Fluorometric Alanine Standard Curve



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