EnzyChrom™ L-Alanine Assay Kit (EALA-100)

Quantitative Colorimetric/Fluorimetric L-Alanine Determination

DESCRIPTION

ALANINE, a nonessential amino acid, is 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. Alanine concentration may have some correlation with high blood pressure, energy intake, cholesterol levels and body mass index.

BioAssay Systems' Alanine Assay Kit provides a simple, direct and automation-ready procedure for measuring alanine concentration. Alanine is converted into pyruvate which can then be directly measured. The color intensity of the reaction product at 570 nm or fluorescence intensity at $\lambda_{em/ex}$ = 585/530 nm is directly proportional to the alanine concentration in the sample.

KEY FEATURES

Sensitive and accurate. Linear detection range in 96-well plate: 1 to 200 µM alanine for colorimetric assays and 0.4 to 20 µM for fluorimetric assays.

APPLICATIONS

Direct Assays: alanine levels in plasma, serum, urine, tissue and culture media.
Drug Discovery/Pharmacology: effects of drugs on alanine metabolism.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Developer:	6 mL	**ALT Enzyme:**	120 µL
Dye Reagent:	120 µL	**Cosubstrate:**	600 µL
Alanine Standard:	400 µL		

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Important:: equilibrate Developer to desired assay temperature. The assay requires 30 min when performed at 37°C or 60 min if performed at RT (25°C).

Sample Preparation

Tissue or cell samples (2×10^6) can be homogenized in 100 µL PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay.

Serum should either be diluted at least 10-fold in dH_2O or deproteinated using a 10 kDa spin filter (e.g. Microcon YM-10). If planning to measure alanine in culture media, if possible avoid media with high pyruvate concentrations (DMEM, L-15, F12, etc.).

Colorimetric Procedure

1. *Standards*. First dilute the Alanine Standard to 200 µM by mixing 5 µL 20 mM Standard with 495 µL dH_2O. Next, dilute standards in 1.5-mL centrifuge tubes as described in the Table. *If assaying culture media with phenol red, dilute the Alanine Standard in culture media*.

No	Premix + dH_2O	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

Transfer 50 µL of each Standard to separate wells in a 96 well plate.

2. *Alanine Detection*. Prepare enough working reagent (WR) for 4 standards and all samples. For each reaction combine the following: 50 µL Developer, 1 µL ALT Enzyme, 5 µL Cosubstrate and 1 µL Dye Reagent. Add 50 µL of WR to each Standard and Sample well. Mix well and incubate protected from light for 30 min at 37°C or 60 min at RT.

3. Read OD_{570nm}.

Fluorimetric Procedure

For fluorimetric assays, the linear detection range is 0.4 to 20 µM alanine. Dilute the Standards prepared in Colorimetric Procedure 1:10 in dH_2O.

Transfer 50 µL standards and 50 µL samples into separate wells of a *black* 96-well plate.

Add 50 µL Working Reagent (see *Colorimetric Procedure*). Tap plate to mix.

Incubate 30 min at 37°C or 60 min at RT and read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the alanine concentration of the Sample,

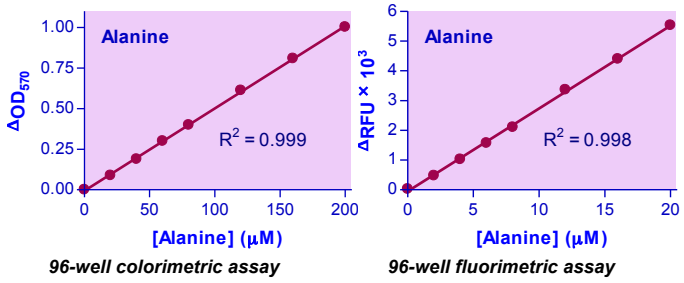
$$[\text{Alanine}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \qquad (\mu M)$$

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the calculated alanine concentration is higher than 200 µM for the colorimetric assay or higher than 20 µM for the fluorimetric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor *n*.

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

Alanine Standard Curves



96-well colorimetric assay ***96-well fluorimetric assay***

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, clear or black flat-bottom 96-well plates, plate reader or centrifuge tubes.

LITERATURE

1. Kiely, A. et al (2007). Pro-inflammatory cytokines increase glucose, alanine and triacylglycerol utilization but inhibit insulin secretion in a clonal pancreatic β-cell line. Journal of Endocrinology 195:113-23.

2. Layman, DK (2003). The Role of Leucine in Weight Loss Diets and Glucose Homeostasis J. Nutr. 133: 261S–267S.

3. Hansen JL, Freier EF. (1978). Direct assays of lactate, pyruvate, beta-hydroxybutyrate, and acetoacetate with a centrifugal analyzer. Clin Chem. 24(3):475-9.