

## 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  $\alpha$ -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  $\mu$ M alanine for colorimetric assays and 0.4 to 20  $\mu$ M 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  $\mu$ L  
**Dye Reagent:** 120  $\mu$ L      **Cosubstrate:** 600  $\mu$ L  
**Alanine Standard:** 400  $\mu$ L

**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** ( $2 \times 10^6$ ) can be homogenized in 100  $\mu$ L 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<sub>2</sub>O 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

- Standards.** First dilute the Alanine Standard to 200  $\mu$ M by mixing 5  $\mu$ L 20 mM Standard with 495  $\mu$ L dH<sub>2</sub>O. 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 <sub>2</sub> O	Alanine ( $\mu$ M)
1	200 $\mu$ L + 0 $\mu$ L	200
2	120 $\mu$ L + 80 $\mu$ L	120
3	60 $\mu$ L + 140 $\mu$ L	60
4	0 $\mu$ L + 200 $\mu$ L	0

Transfer 50  $\mu$ L of each Standard to separate wells in a 96 well plate.

- Alanine Detection.** Prepare enough working reagent (WR) for 4 standards and all samples. For each reaction combine the following: 50  $\mu$ L Developer, 1  $\mu$ L ALT Enzyme, 5  $\mu$ L Cosubstrate and 1  $\mu$ L Dye Reagent. Add 50  $\mu$ L 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.

- Read OD<sub>570nm</sub>.

##### Fluorimetric Procedure

For fluorimetric assays, the linear detection range is 0.4 to 20  $\mu$ M alanine. Dilute the Standards prepared in Colorimetric Procedure 1:10 in dH<sub>2</sub>O.

Transfer 50  $\mu$ L standards and 50  $\mu$ L samples into separate wells of a black 96-well plate.

Add 50  $\mu$ L Working Reagent (see *Colorimetric Procedure*). Tap plate to mix.

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

#### CALCULATION

Subtract the blank value (#4) from the standard values and plot the  $\Delta$ OD or  $\Delta$ F against standard concentrations. Determine the slope and calculate the alanine concentration of the Sample,

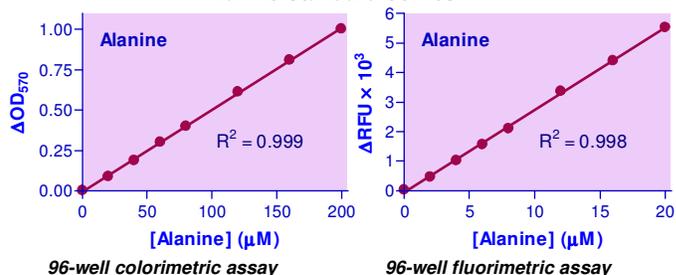
$$[\text{Alanine}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

$R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  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  $\mu$ M for the colorimetric assay or higher than 20  $\mu$ M for the fluorimetric assay, dilute sample in dH<sub>2</sub>O and repeat assay. Multiply result by the dilution factor  $n$ .

**Conversions:** 1 mg/dL alanine equals 112.2  $\mu$ M, 0.001% or 10 ppm.

#### Alanine Standard Curves



#### MATERIALS REQUIRED, BUT NOT PROVIDED

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

#### LITERATURE

- Kiely, A. et al (2007). Pro-inflammatory cytokines increase glucose, alanine and triacylglycerol utilization but inhibit insulin secretion in a clonal pancreatic  $\beta$ -cell line. *Journal of Endocrinology* 195:113-23.
- Layman, DK (2003). The Role of Leucine in Weight Loss Diets and Glucose Homeostasis. *J. Nutr.* 133: 261S-267S.
- Hansen JL, Freier EF. (1978). Direct assays of lactate, pyruvate, beta-hydroxybutyrate, and acetoacetate with a centrifugal analyzer. *Clin Chem.* 24(3):475-9.

