EnzyChrom™ Alanine Transaminase Assay Kit (Cat# EALT-100)
Colorimetric Determination of Alanine Transaminase activity

DESCRIPTION
Alanine Transaminase (ALT), also known as serum alanine aminotransferase (ALAT) or pyruvic transaminase (SGPT), facilitates the conversion of alanine and α-ketoglutarate to pyruvate and glutamate. ALT plays an important role in gluconeogenesis and amino acid metabolism. ALT is found mainly in the liver, and, to a lesser extent, in kidney, heart, muscle, and pancreas tissues. Normal serum levels of ALT are low, and increased serum ALT activity is widely used as a marker for liver damage.

Simple, direct and automation-ready procedures for measuring ALT activity find wide applications in research and drug discovery. BioAssay Systems’ ALT activity assay is based on the quantification of pyruvate produced by ALT. In this assay, pyruvate and NADH are converted to lactate and NAD by the enzyme lactate dehydrogenase (LDH). The decrease in NADH absorbance at 340 nm is proportional to ALT activity.

KEY FEATURES
Sensitive. Linear detection range: 2–100 U/L.
Simple and convenient. This simple, convenient assay can be carried out in a microplate or a cuvette and takes only 10 min.

APPLICATIONS
Direct Assays: ALT activity in serum, plasma and other biological samples.
Drug Discovery/Pharmacology: effects of drugs on ALT activity.

KIT CONTENTS
- Assay Buffer: 24 mL
- LDH: 120 µL
- Cosubstrate: 600 µL
- NADH: 500 µL

Storage conditions: The kit is shipped on ice. Store all reagents at -20°C. Shelf life of three 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.

ASSAY PROCEDURES
Equilibrate all components to room temperature. Mix assay buffer well by vigorous shaking. Keep thawed enzyme on ice.

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.

Assay is compatible with serum or plasma (heparin, EDTA). Samples should be clear and free of particles or precipitates. Hemolyzed samples should not be used.

Procedure using cuvettes
1. For each assay, include one Standard and one Blank control.

For each Sample and Standard, prepare Working Reagent by mixing 1000 µL Assay Buffer, 25 µL Cosubstrate, 5 µL Enzyme Mix and 20 µL NADH. Transfer 1000 µL Working Reagent to each sample cuvette and standard cuvette. Warm to desired temperature (e.g. 37°C).

For Blank control, mix 1000 µL Assay Buffer, 25 µL Cosubstrate, 5 µL Enzyme Mix and 20 µL H₂O. Transfer 1000 µL Reagent to the Blank control cuvette. Warm to desired temperature (e.g. 37°C).

2. Prewarm sample to the desired temperature. Add 100 µL Sample to the Sample Cuvette. Transfer 100 µL H₂O to the Standard Cuvette and to Blank Control cuvette, respectively. Mix immediately.

3. Read OD₃₄₀nm at 5 min and 10 min. Alternatively, record kinetics at 340 nm.

CALKULATION
For each Sample, calculate the rate of NADH consumption by subtracting the OD at 10 min from the OD at 5 min (∆OD₅). Similarly, calculate the rate (∆OD₅) for the NADH standard (OD₅–10min).

Determine ALT activity using the following equation,

\[ ALT = \frac{381 \times (\Delta OD₅)}{OD_{STD} - OD_{BLK}} \ (U/L) \]

\( OD_{STD} \) and \( OD_{BLK} \) are the \( OD_{340nm} \) values of NADH Standard and Blank at 5 min, respectively. The factor 381 is derived from

\[ \text{Factor} = \frac{10 \text{ mM NADH} \times \frac{4 \text{ µL Vol}_{\text{wr}}}{210 \text{ µL Vol}_{\text{wr}}}}{220 \text{ µL Vol}_{\text{wr}}} \times \frac{11 \text{ (sample dilution)}}{5 \text{ min}} = 381 \text{ µM/min} \]

If the calculated ALT activity is higher than 100 U/L, dilute sample in Assay Buffer and repeat assay. Multiply results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED
Pipeting devices and accessories. Clear bottom 96-well plates (e.g. Corning Costar) and plate reader or spectrophotometer and cuvettes for measuring OD₃₄₀nm.

LITERATURE
