Product Manual

Alanine Assay Kit

Catalog Number

MET-5093 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Alanine is a non-essential, non-polar amino acid that has a key metabolic role in the glucose alanine cycle between the liver and tissues. In tissues such as muscle, amino acids are broken down to provide energy; during the process of transamination amino groups are collected in the form of glutamate. Amino groups are then transferred to pyruvate from glutamate by alanine aminotransferase through the glycolysis cycle, which results in the formation of alanine and α-ketoglutarate. Alanine is transported to the liver through the bloodstream. In the liver, alanine aminotransferase performs the reversible reaction consuming alanine and regenerating pyruvate to be used in gluconeogenesis (the glucose produced returns to muscle tissue through the circulation system). Changes in the alanine cycle that increase serum alanine aminotransferase (ALT) levels have been linked to the development of type II diabetes or liver fibrosis in chronic hepatitis B infected patients.

Assay Principle

The Alanine Assay Kit is a sensitive, quantitative colorimetric assay for alanine. The provided reagents are sufficient for the evaluation of 200 assays. The unknown samples or Alanine standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, an electron mediator, and L-Alanine Dehydrogenase (ADH). During a brief incubation, the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of Alanine in the unknown samples is determined by comparison with a predetermined Alanine standard curve.

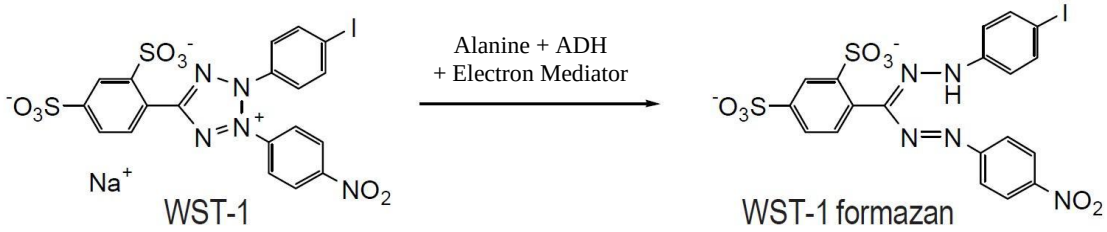

Figure 1. Alanine Assay Principle.

Note: Each sample replicate requires 2 assays, one treated with L-alanine dehydrogenase (+ADH) and one without (-ADH). Alanine levels are calculated from the difference in OD readings from the 2 wells.

Related Products

1. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5070: Glycine Assay Kit
4. MET-5073: Tyrosine Assay Kit
5. MET-5080: Glutamate Assay Kit (Colorimetric)

Kit Components

1. 20X Colorimetric Probe (Part No. 50934C): Two 1 mL amber vials.
2. L-Alanine Standard (Part No. 50931C): One 100 µL vial at 25 mM.
3. 10X Assay Buffer (Part No. 50932A): One 30 mL bottle.
4. 50X NAD+ (Part No. 50803D): One 800 µL vial.
5. L-Alanine Dehydrogenase (100X) (Part No. 50933D): One 400 µL vial at 62.5 U/mL

Note: 1 U corresponds to the amount of enzyme which converts 1 µmol L-alanine per minute at pH 10.0 and 30°C (NAD as cofactor).

Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate

Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Store the 50X NAD+ at -80°C. Store all remaining components at -20ºC. The 20X Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Dilute the 20X Colorimetric Probe, the L-Alanine Dehydrogenase (100X) and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 200 µL of 20X Colorimetric Probe, 40 µL of Alanine Dehydrogenase (100X), and 80 µL of 50X NAD+ to 3.68 mL of 1X Assay Buffer.

  Note: Scale down the described example appropriately and prepare only enough for immediate use.
- Control Mix: Dilute both the 20X Colorimetric Probe and the 50X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 20 assays add 200 µL of 20X Colorimetric Probe, and 80 µL of 50X NAD+ to 3.72 mL of 1X Assay Buffer.

  Note: Scale down the described example appropriately and prepare only enough for immediate use.

Preparation of Samples

Notes: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with unknown samples.

- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary. Prepare the Alanine standard curve in the same non-conditioned media.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or diluted as necessary in deionized water.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in deionized water.

Preparation of Standard Curve

Prepare fresh Alanine standards according to Table 1.

Standard Tubes	25 mM Alanine Solution (µL)	1X Assay Buffer (µL)	Alanine (µM)
1	10	490	500
2	250 of Tube #1	250	250
3	250 of Tube #2	250	125
4	250 of Tube #3	250	62.5
5	250 of Tube #4	250	31.25
6	250 of Tube #5	250	15.63
7	250 of Tube #6	250	7.81
8	0	250	0

Table 1. Preparation of Alanine Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with ADH (Reaction Mix) and one without the enzyme (Control Mix) to measure endogenous sample background.
2. Add 50 µL of each sample (Alanine standard or unknown) into wells of a 96 well plate.
3. Add 200 µL of Reaction Mix to the standards and to one half of the paired sample wells and mix the well contents thoroughly.
4. Add 200 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate at 37°C for 15 minutes.
6. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Alanine Assay (Colorimetric) results. One should use the data below for reference only. This data should not be used to interpret actual results.

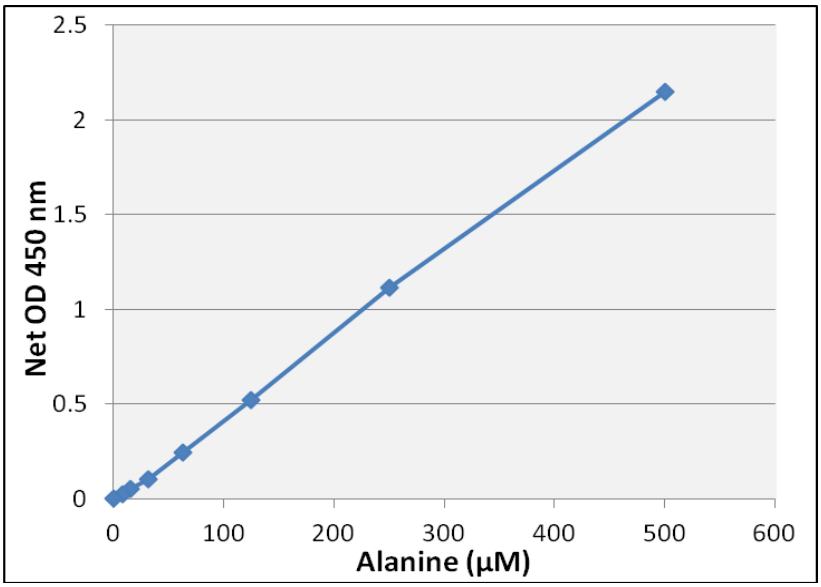

Figure 2. Alanine Standard Curve.

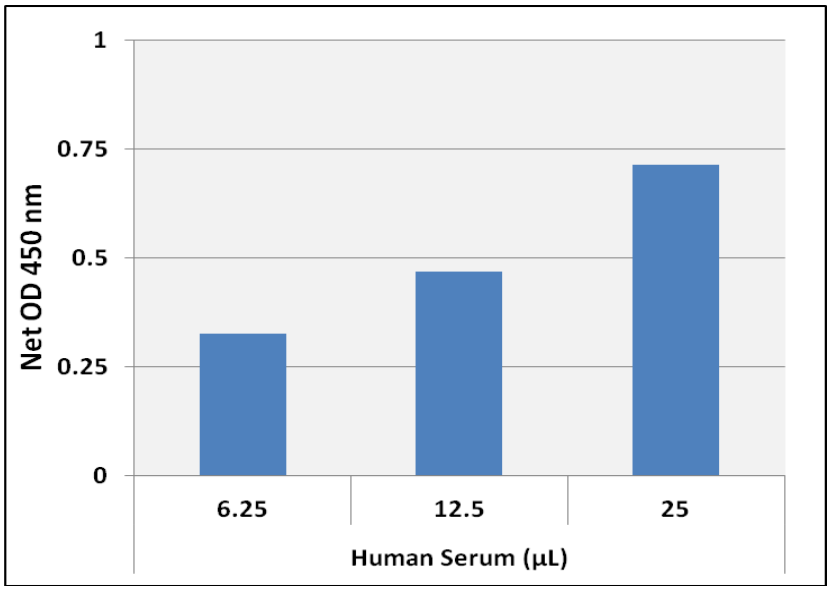

Figure 3. Detection of Alanine in Serum. Human serum was assayed according to the kit protocol.

Calculation of Results.

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without ADH (-ADH) from the sample well values containing enzyme (+ADH) to obtain the difference. The absorbance difference is due to the enzyme ADH activity:

$$\Delta A = A_{(+ADH)} - A_{(-ADH)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of alanine present in the sample. Only use values within the range of the standard curve.

References

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4. Lee SH, Cho DY, Joo NS, Kim KM, Kim KN (2018) *Turk J Gastroenterol*. **29**:52-59
5. Hatazawa Y, Qian K, Gong DW, Kamei Y (2018) *PLoS One*. **13**: e0190904
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Recent Product Citation

Lukić, J. et al. (2021). Enrichment of Larval Fish Feed with Free Amino Acids and Proteins by Coating with Lactobacillus paracasei subsp. paracasei BGHN14 Homogenate. *Turkish J. Fish. Aquat. Sci*. **21**(11):569-573. doi: 10.4194/1303-2712-v21_11_05.

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