Alanine Assay kit

ARG83374 Alanine Assay kit is an assay kit for Alanine in Serum, plasma, urine, Cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83374

Package: 200 assay

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

L-alanine is the L-enantiomer of alanine. It has a role as an EC 4.3.1.15 (diaminopropionate ammonia-lyase) inhibitor and a fundamental metabolite. It is a pyruvate family amino acid, a proteinogenic amino acid, a L-alpha-amino acid and an alanine. It is a conjugate base of a L-alaninium. It is a conjugate acid of a L-alaninate. It is an enantiomer of a D-alanine. It is a tautomer of a L-alanine zwitterion.

PRINCIPLE OF THE ASSAY

ARG83374 Alanine Assay Kit measures Alanine content in biological samples. Samples or Alanine standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, electron mediator, and Alanine Dehydrogenase. During a brief incubation, the WST-1 is converted to the formazan. Samples are compared to a known concentration of Alanine standard. The intensity of the color is measured at a wavelength of 450 nm. The concentration of Alanine in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 10X Assay Buffer at RT, 50X NAD+ at -80°C

Store other component at ≤ -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (25 mM)	100 µL	-20°C
10X Assay Buffer	30 mL	RT
50X NAD+	800 µL	-80°C
L-Alanine Dehydrogenase	400 µL	-20°C
Probe	1 mL X 2	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Flat bottomed 96-well black microplate and tube.
- 1X PBS and deionized water
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Upon received, store 10X Assay Buffer at RT, 50X NAD+ at -80°C Store other component at ≤ -20°C. Use the kit before expiration date Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at RT.
- **Reaction Mix:** Dilute the Probe at 1:20, L-Alanine Dehydrogenase at 1:100 and NAD+ at 1:50 in 1X Assay Buffer. For 20 assays, add 200 µL Probe, 40 µL Alanine Dehydrogenase and 80 µL of 50X NAD+ to 3.68 mL of 1X Assay Buffer for a total of 4 mL. Store the Reaction Mix at 4°C for 1 day
- **Control Mix:** Dilute the Probe at 1:20 and NAD+ at 1:50 in 1X Assay Buffer. For 20 assays, add 200 µL Probe and 80 µL of 50X NAD+ to 3.72 mL of 1X Assay Buffer for a total of 4 mL. Store the Reaction Mix at 4°C for 1 day.
- **Standards**: Add 5 µl of 2.0 mM stock standard into 495 µl 1X Assay Buffer to generate a standard with 20 µM of Tyrosine. Dilute the standards with 1X Assay Buffer serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	Alanine (µM)	Volume of 1X Assay Buffer (µL)	Volume of Alanine (µL)
S1	500	490	10 (25 mM stock)
S2	250	250	250(S1)
S3	125	250	250(S2)
S4	62.5	250	250(S3)
S5	31.25	250	250(S4)
S6	15.63	250	250(S5)
S7	7.81	250	250(S6)
S8	0	250	0

ASSAY PROCEDURE

Each samples should be assayed in at least duplicates, one to be treated with L-Alanine Dehydrogenase and one without, to measure endogenous background.

1. Add 50 µl of standard and sample to each wells.
2. Add 200 µL of Reaction Mix to standard and half of sample wells.
3. Add 200 µL of Control Mix to other half of sample wells.
4. Mix well and Incubate for 15 min at 37°C.
5. Read O.D. with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

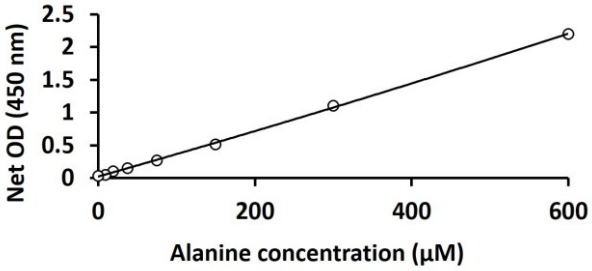
1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average value of Standard 0 from all standard value.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Subtract the sample well values without L-Alanine Dehydrogenase from the sample well values containing L-Alanine Dehydrogenase (Control Mix) to obtain the difference. The absorbance difference is due to the L-Alanine Dehydrogenase activity:

   $\Delta A = A_{\text{Reaction Mix}} - A_{\text{Control Mix}}$

5. Compare the change in absorbance Δ A of each sample to the standard curve to determine and extrapolate the quantity of bile acid present in the sample. Only use values within the range of the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Alanine ranged from 7.81-500 µM. The mean MDD was 3.0 µM