NOTE: Revisions to "Preparation of Reagents" and "Assay Protocol"

Product Manual

L-Amino Acid Assay Kit (Fluorometric)

Catalog Number

MET-5055 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Amino acids are organic compounds that contain amine (-NH2) and carboxyl (-COOH) functional groups, as well as a side-chain (R group) which confers uniqueness to each amino acid. The main elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, although other elements can be found in some amino acids. About 500 amino acids are known, however, only 20 are coded in the human genome. Amino acids are the monomers which are joined together to make short polymer chains called peptides or longer chains called proteins. Non-protein amino acids play important roles in the formation of biologically important molecules. For example, tryptophan is processed into the neurotransmitter serotonin, while tyrosine (and its precursor phenylalanine) is processed into neurotransmitters dopamine, epinephrine and norepinephrine.

When consumed and absorbed by the human body, the standard amino acids are used to make proteins and other molecules or are oxidized to urea and carbon dioxide to be used as a form of energy. The oxidation pathway begins with transamidase removal of the amino group, and this group is then processed through the urea cycle. The other transamidation product is a keto acid that is used for the citric acid cycle. Through the process of gluconeogenesis, some amino acids can also be converted into glucose. Out of the standard 20 amino acids, nine amino acids (His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val), are considered to be essential amino acids because the human body cannot make them from other molecules in enough amounts needed for normal growth, so they must be obtained from food sources.

Cell Biolabs' L-Amino Acid Assay Kit is a simple fluorometric assay that measures the amount of total free L-Amino Acids (except for Glycine) present in foods or biological samples in a 96-well microtiter plate format. Amino Acids in polypeptide chains (peptides and proteins) are not detected. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, L-Alanine standards and unknown samples. Sample L-Amino Acid concentrations are determined by comparison with a known L-Alanine standard. The kit has a detection sensitivity limit of 6.25 µM L-Amino Acids.

Assay Principle

Cell Biolabs' L-Amino Acid Assay Kit measures L-Amino Acids within food or biological samples. An L-amino acid is oxidatively deaminated by L-Amino Acid oxidase into its corresponding α-keto acid plus ammonia and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are read with a standard 96-well fluorometric plate reader. Samples are compared to a known concentration of L-Alanine standard within the 96-well microtiter plate format (Figure 1).

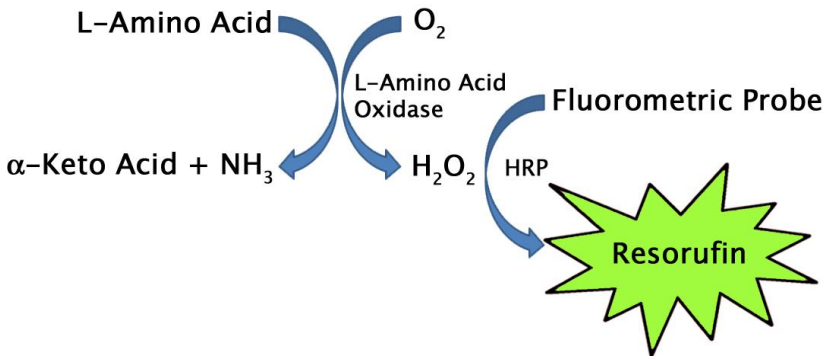

Figure 1. L-Amino Acid Assay Principle.

Related Products

1. MET-5056: Branched Chain Amino Acids (Colorimetric)
2. MET-5029: Pyruvate Assay Kit (Fluorometric)
3. MET-5070: Glycine Assay Kit (Fluorometric)
4. MET-5129: Lysine Assay Kit (Fluorometric)
5. STA-674: Glutamate Assay Kit (Fluorometric)

Kit Components (shipped on blue ice)

1. L-Alanine Standard (Part No. 50541C): One 30 µL tube at 100 mM.
2. 1X Assay Buffer (Part No. 50542A): One 25 mL bottle.
3. Fluorometric Probe (Part No. 50231C): One 50 µL amber tube.
4. HRP (Part No. 234402): One 100 µL tube at 100 U/mL in glycerol
5. L-Amino Acid Oxidase (Part No. 50544B): One 170 µL tube at 32 U/mL

Note: One unit is defined as the amount of enzyme that will oxidatively deaminate 1.0 µmole of L-leucine per minute at pH 6.5 and 25°C.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. 96-well black or fluorescence-compatible microtiter plate

Storage

Upon receipt, store the L-Alanine Standard, Fluorometric Probe, and HRP at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles. Store the L-Amino Acid Oxidase at 4°C **(DO NOT FREEZE L-Amino Acid Oxidase)**. Store the 1X Assay Buffer at room temperature.

Preparation of Reagents

- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, and L-Amino Acid Oxidase 1:30 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, and 33.3 µL of L-Amino Acid Oxidase to 954.7 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- 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 may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer.

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 samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh L-Alanine standards before use by diluting in 1X Assay Buffer according to Table 2 below.

Standard Tubes	100 mM L-Alanine Solution (µL)	1X Assay Buffer (µL)	L-Alanine (µM)
1	5	1245	400
2	250 of Tube #1	250	200
3	250 of Tube #2	250	100
4	250 of Tube #3	250	50
5	250 of Tube #4	250	25
6	250 of Tube #5	250	12.5
7	250 of Tube #6	250	6.25
8	0	250	0

Table 2. Preparation of L-Amino Acid 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.
2. Add 50 µL of each L-Alanine standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 180 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of L-Amino Acids within samples by comparing the sample fluorescence to the standard curve.

Example of Results

The following figures demonstrate typical L-Amino Acid Assay Kit (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

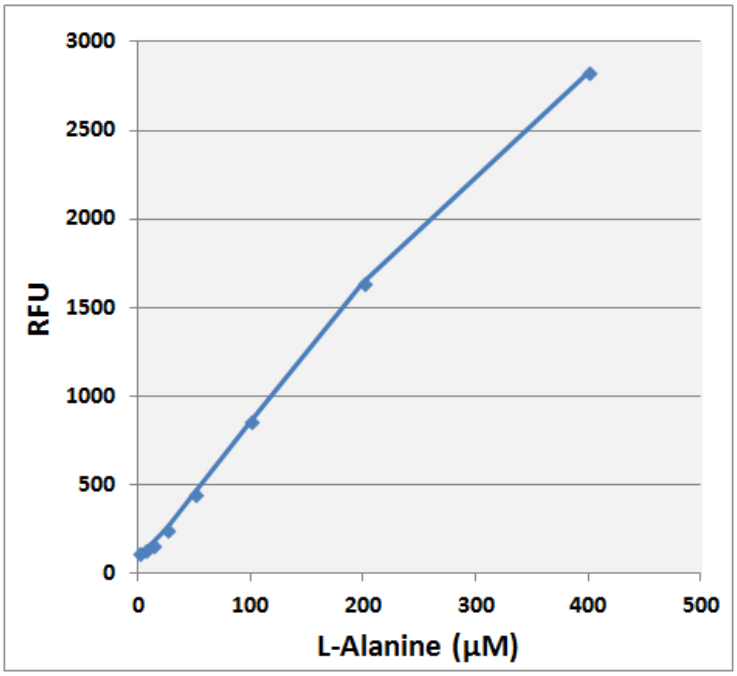

Figure 2: L-Alanine standard curve.

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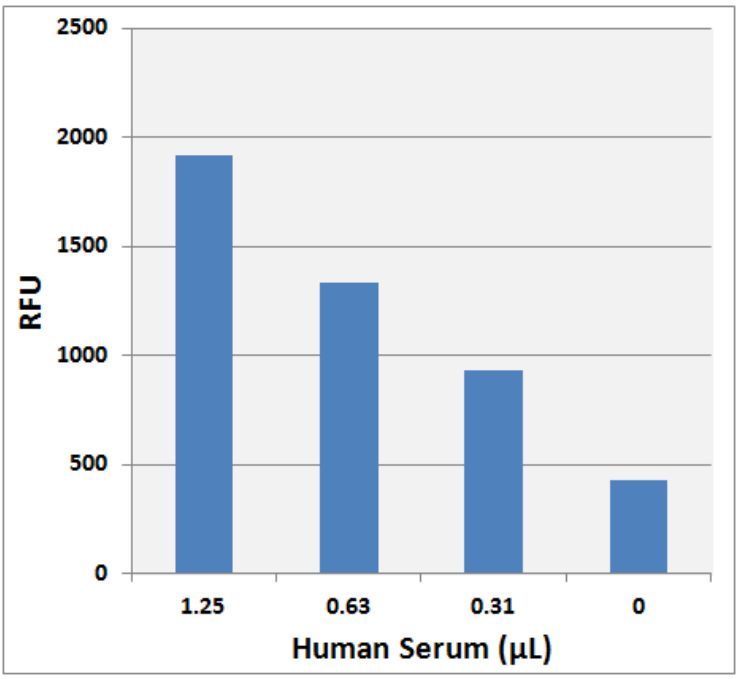

Figure 3: L-Amino Acid detection in human serum using the L-Amino Acid Assay Kit (Fluorometric).

References

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