Product Manual

L-Amino Acid Assay Kit (Colorimetric)

Catalog Number
MET-5054 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures
**Introduction**

Amino acids are organic compounds that contain amine (\(-\text{NH}_2\)) and carboxyl (\(-\text{COOH}\)) 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, but 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) are 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 colorimetric assay that measures the total amount of 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 62.5 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 colorimetric 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 colorimetric 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-5053: Total Thiol Assay Kit (Colorimetric)
2. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
3. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
4. STA-670: Homocysteine ELISA Kit
5. STA-674: Glutamate Assay Kit
6. STA-675: Hydroxyproline Assay Kit
7. STA-680: Glucose Assay Kit (Colorimetric)
8. STA-681: Glucose Assay Kit (Fluorometric)
9. STA-682: Total Carbohydrate Assay Kit

Kit Components
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. Colorimetric Probe (Part No. 50122C): 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. Microcentrifuge tubes
4. 10 μL to 1000 μL adjustable single channel micropipettes with disposable tips
5. 50 μL to 300 μL adjustable multichannel micropipette with disposable tips
6. Standard 96-well clear microtiter plate and/or clear cell culture microplate
7. Multichannel micropipette reservoir
8. Spectrophotometric microplate reader capable of reading in the 540-570 nm range

**Storage**

Upon receipt, store the L-Alanine Standard, Colorimetric Probe, and HRP at -20°C. The Colorimetric 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 Colorimetric Probe 1:100, HRP 1:500, and L-Amino Acid Oxidase 1:30 in 1X Assay Buffer. For example, add 10 μL Colorimetric 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 4°C.

  *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 10⁶ 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 Colorimetric 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 Colorimetric 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.

<table>
<thead>
<tr>
<th>Standard Tubes</th>
<th>100 mM L-Alanine Solution (µL)</th>
<th>1X Assay Buffer (µL)</th>
<th>L-Alanine (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>495</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>250 of Tube #1</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>250 of Tube #2</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>4</td>
<td>250 of Tube #3</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>250 of Tube #4</td>
<td>250</td>
<td>62.5</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>250</td>
<td>0</td>
</tr>
</tbody>
</table>

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 spectrophotometric microplate reader in the 540-570 nm range.
5. Calculate the concentration of L-Amino Acids within samples by comparing the sample OD to the standard curve.

**Example of Results**
The following figures demonstrate typical L-Amino Acid Assay Kit (Colorimetric) 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.
Figure 3: L-Amino Acid detection in human serum using the L-Amino Acid Assay Kit (Colorimetric).

References

Warranty
These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS’s sole obligation and purchaser’s
exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information
Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2017-2021: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.