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Datasheet for ABIN5067586 L-Amino Acid Assay Kit (Colorimetric)

3 Images



Overview

Quantity:	100 tests
Application:	Biochemical Assay (BCA)
Product Details	
Purpose:	L-Amino Acid Assay Kit measures L-Amino Acids within food or biological samples.
Detection Method:	Colorimetric
Sensitivity:	62.5 μM
Characteristics:	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

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	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 μ
	M L-Amino Acids.
Components:	1. L-Alanine Standard : One 30 μL tube at 100 mM.
	2. 1X Assay Buffer : One 25 mL bottle.
	3. Colorimetric Probe : One 50 µL amber tube.
	4. HRP : One 100 μL tube at 100 U/mL in glycerol
	5. L-Amino Acid Oxidase : Two 1.25 mL tubes at 1 U/mL Note: One unit is defined as the
	amount of enzyme that will oxidatively deaminate 1.0 μ mole of L- phenylalanine per minute

at pH 6.5 and 37°C. 3

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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, 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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Protocol:	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 .
Reagent Preparation:	• Reaction Mix: Prepare a Reaction Mix by diluting the Colorimetric Probe 1:100, HRP 1:500,
	and L- Amino Acid Oxidase 1:2 in 1X Assay Buffer. For example, add 10 μ L Colorimetric Probe
	stock solution, 2 μL HRP stock solution, and 0.5 mL of L-Amino Acid Oxidase to 488 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.

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Sample Preparation:	 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 µ M will oxidize the Colorimetric Probe and could result in erroneous readings. To minimize this 4 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).
Assay Procedure:	 Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate. Add 50 µL of each L-Alanine standard or unknown sample into wells of a 96-well microtiter plate. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 90 minutes at 37 °C protected from light. Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics. Read the plate with a spectrophotometric microplate reader in the 540-570 nm range. Calculate the concentration of L-Amino Acids within samples by comparing the sample OD to the standard curve.
Restrictions:	For Research Use only
Handling	
Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	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.

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Biochemical Assay

Image 1. L-Alanine standard curve.

Biochemical Assay

Image 2. L-Amino Acid detection in human serum using the L-Amino Acid Assay Kit (Colorimetric).



