

Phenylalanine Assay Kit (Fluorometric)

LS-K313-100 (100 Tests) • See Storage Conditions Below



Introduction

L-Phenylalanine is one of the twenty common amino acids and an important precursor for several key signal molecules such as dopamine, norepinephrine, epinephrine, and the skin pigment melanin. It is found naturally in the breast milk of mammals, and used as nutritional supplements in food and drink products. The genetic disorder phenylketonuria is the inability to metabolize phenylalanine. Individuals with this disorder are known as "phenylketonurics". Individuals who cannot metabolize phenylalanine must monitor their intake of protein to control the buildup of phenylalanine. This L-Phenylalanine Assay Kit provides a convenient fluorometric means to measure L-phenylalanine in biological samples. In the assay, L-phenylalanine is oxidized by phenylalanine dehydrogenase, producing NADH, which reduces a fluorescent dye to a highly fluorescent product. The resulting fluorescence intensity ($\lambda_{ex/em} = 530/585 \text{ nm}$) is linear to the L-phenylalanine concentration in the sample.

Key Features

- Safe. Non-radioactive assay.
- Sensitive and accurate. Linear detection range of 2 - 300 μM L-phenylalanine.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Determination of L-phenylalanine in serum, urine and other biological samples.

Components

Component	K313-100
	100 Tests
Assay Buffer	10 mL
NAD Solution	1 mL
Probe	750 μL
Enzyme A	Dried
Enzyme B	120 μL
Standard	120 μL

Materials Not Supplied

Pipetting devices, centrifuge tubes, black flat-bottom 96-well plates and plate reader.

Storage

The kit is shipped on ice. Store all reagents at -20°C except for reconstituted Enzyme A. Shelf life of 6 months after receipt.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Phenylalanine Assay Kit (Fluorometric)

LS-K313-100 (100 Tests) • See Storage Conditions Below



Assay Procedure

Reagent Preparation

Reconstitute Enzyme A by adding 120 μL Assay Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at 4°C (DO NOT FREEZE) and use within 1 month.

Procedure

Use black flat-bottom plates. Prior to assay, bring all reagents to room temperature. Briefly centrifuge enzyme tubes, keep on ice during assay.

- Standards. Prepare 400 μL 300 μM L-Phenylalanine Premix by mixing 6 μL 20 mM Standard and 394 μL dH₂O. Dilute standard as follows.

No	Premix + H ₂ O	Standard (μM)
1	90 μL + 0 μL	300
2	60 μL + 30 μL	200
3	30 μL + 60 μL	100
4	0 μL + 100 μL	0

Transfer 10 μL standards into separate wells of the plate.

- Sample. Liquid samples can be assayed directly. Tissue (20 mg) or cells (2×10^6) can be homogenized in 200 μL ice-cold PBS, followed by centrifugation at 14,000 rpm for 5 min. Use clear supernatant for assay. Samples not measured on the same day can be stored frozen, preferably at -80°C.

Transfer 10 μL of each sample in duplicate, one for Sample and one for Sample Blank, to separate wells of the plate.

- Assay. For standards and sample wells, prepare enough Working Reagent, for each well, by mixing 85 μL Reagent A, 8 μL NAD, 5 μL Probe, 1 μL reconstituted Enzyme A and 1 μL Enzyme B.

For the Sample Blank wells, prepare Blank Reagent for each well by mixing 86 μL Reagent A, 8 μL NAD, 5 μL Probe and 1 μL Enzyme B (i.e. NO Enzyme A).

- Add 90 μL Working Reagent to Standard and Sample wells, and 90 μL Blank Reagent to the Sample Blank wells. Tap plate to mix. Incubate for 20 min in the dark.
- Read fluorescence intensity at $\lambda_{\text{exc/em}} = 530/585$ nm.

Calculations

Plot the L-phenylalanine Standard Curve and determine its Slope. Phenylalanine concentration of a Sample is calculated as

$$[\text{L-Phenylalanine}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{M})$$

where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and Sample blank, respectively. Slope is the slope of the standard curve.

Note: if the Sample L-phenylalanine concentration is higher than 300 μM , dilute sample in water and repeat the assay. Multiply result by the dilution factor.

Conversion factor: 1 μM L-phenylalanine is equivalent to 165 $\mu\text{g/L}$ or 165 ppb.

FOR RESEARCH USE ONLY! Not for use in humans.

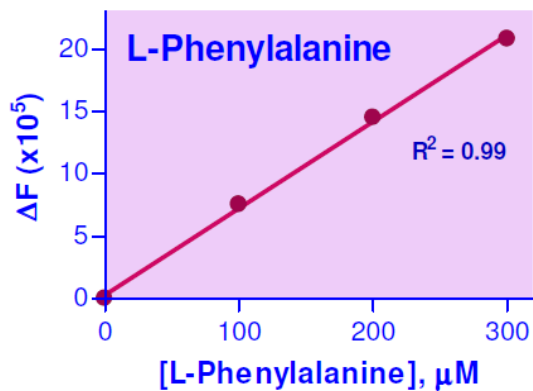
LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Phenylalanine Assay Kit (Fluorometric)

LS-K313-100 (100 Tests) • See Storage Conditions Below

Sample Data



L-Phenylalanine Standard Curve

Version: V.08.09.2018

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com