EnzyChrom™ Phenylalanine Assay Kit (EPHE-100)

Quantitative Fluorimetric Determination of L-Phenylalanine

DESCRIPTION

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.

BioAssay Systems' L-Phenylalanine Assay Kit provides a convenient fluorimetric 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 ($\lambda_{exc/em}$ = 530/585 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.

KIT CONTENTS

Assay Buffer:	10 mL	**Enzyme A:**	Dried
NAD Solution:	1 mL	**Enzyme B:**	120 µL
Probe:	750 µL	**Standard:**	120 µL

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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.

Assay Procedure:

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

1. *Standards*. Prepare 400 µL 300 µM L-Phenylalanine Premix by mixing 6 µL 20 mM Standard and 394 µL dH_2O. Dilute standard as follows.

No	Premix + H_2O	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.

2. *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.

3. *Assay*. For standards and sample wells, prepare enough Working Reagent, for each well, by mixing 85 µL Assay Buffer, 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 Assay Buffer, 8 µL NAD, 5 µL Probe and 1 µL Enzyme B (i.e. NO Enzyme A).

4. 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.

5. Read fluorescence intensity at $\lambda_{exc/em}$ = 530/585 nm.

CALCULATION

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

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

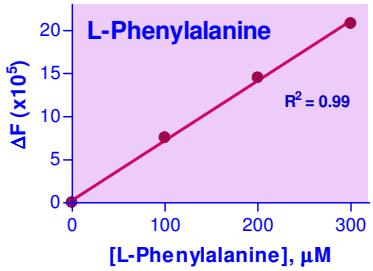
where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and Sample blank, respectively. Slope is the slope of the standard curve.

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 µg/L or 165 ppb.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



L-Phenylalanine Standard Curve

LITERATURE

1. Campbell RS et al (1994). Development of an enzyme-mediated assay for phenylalanine in blood spots. Ann Clin Biochem 31(2):140-6.
2. Hummel W et al (1988). Enzymatic determination of L-phenylalanine and phenylpyruvate with L-phenylalanine dehydrogenase. Anal Biochem 170(2):397-401.
3. Mehrle PM, DeClue ME (1973). Phenylalanine determination in fish serum: adaptation of a mammalian method to fish. Anal Biochem 52(2):660-1.