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.

Sample Preparation: 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.

Samples with a complex biological matrix (e.g. serum, urine) will require dilution, an internal standard, and measurement of the initial rates (i.e. kinetics) of the assay. See *Internal Standard Method*.

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.

Standard Curve Method is used for L-phenylalanine assay in a simple sample matrix, i.e. no or little interferences are expected.

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*. 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 (WR), 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 (WR_o) 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 WR to Standard and Sample wells, and 90 µL WR_o to the Sample Blank wells. Tap plate to mix. Incubate for 20 min in the dark, then read fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm.

5. Plot the L-phenylalanine Standard Curve and determine its Slope. L-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.

Internal Standard Method: Since this is a kinetic assay, it is recommended to: 1). Pre-set plate reader to record fluorescence kinetics at $\lambda_{ex/em}$ = 530/585nm every minute. 2). In order to ensure reactions start at approximately the same time, add/mix reagents quickly and thoroughly, and read plate immediately.

Samples requiring an internal standard will need three separate reactions: (a) Sample plus spike, (b) Sample alone, and (c) Sample Blank. For example, serum samples need to be diluted 1:5 in H_2O (e.g. 10 µL serum + 40 µL H_2O). Transfer 10 µL of diluted sample to two separate wells of a plate for Sample alone (b) and Sample Blank (c) reactions. Serum plus spike (a) can be prepared by diluting the provided 20 mM Phe standard to 1 mM, then mixing 10 µL serum + 35 µL H_2O + 5 µL 1mM Phe standard (results in 1:5 diluted serum with 100 µM internal standard). Transfer 10 µL of this mixture to a separate well for Sample plus spike reaction.

For the internal standard method, follow the same instructions as Step 3 of the *Standard Curve Method* to prepare enough WR for all Sample plus spike and Sample alone wells, and prepare enough WR_o for all Sample Blank wells.

Add 90 µL WR to all Sample plus spike and Sample alone wells, and 90 µL WR_o for all Sample Blank wells. Tap plate to mix, and immediately read fluorescence kinetics every minute at $\lambda_{ex/em}$ = 530/585 nm for 10 min. Use F_{10min} and F_{0min} for calculation.

The sample L-phenylalanine concentration is calculated as follows:

$$[\text{L-Phe}] = \frac{(F_{S10} - F_{S0}) - (F_{O10} - F_{O0})}{(F_{SP10} - F_{SP0}) - (F_{S10} - F_{S0})} \times 100 \times 5\ (\mu M)$$

Where F_{10} and F_0 are fluorescence intensities at time 10 min and 0 min, respectively. S, SP and O denote the Sample alone, Sample plus spike, and Sample Blank wells, respectively. "100" is the spiked L-Phe concentration (100 µM). "5" the sample dilution factor.

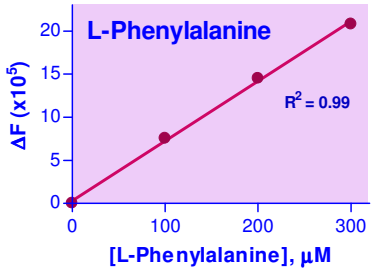
Conversion factor: 1 µM L-phenylalanine is equivalent to 165 µg/L or 165 ppb.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

EXAMPLES

Following the internal standard procedure, L-phenylalanine concentration was found to be 38.7 µM for a human serum sample and 20.2 µM for a rat serum sample. These results are examples and are not intended as expected values.



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.