Phenylalanine Assay Kit (Fluorometric)

Phenylalanine Assay Kit (Fluorometric) is a detection kit for the quantification of Phenylalanine in serum and urine.

Catalog number: ARG82190

Package: 100 tests

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INTRODUCTION

Phenylalanine (symbol Phe or F) is an essential α-amino acid with the formula $C_9H_{11}NO_2$. It can be viewed as a benzyl group substituted for the methyl group of alanine, or a phenyl group in place of a terminal hydrogen of alanine. This essential amino acid is classified as neutral, and nonpolar because of the inert and hydrophobic nature of the benzyl side chain. The L-isomer is used to biochemically form proteins, coded for by DNA. Phenylalanine is a precursor for tyrosine, the monoamine neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline), and the skin pigment melanin. It is encoded by the codons UUU and UUC.

Phenylalanine is found naturally in the breast milk of mammals. It is used in the manufacture of food and drink products and sold as a nutritional supplement for its reputed analgesic and antidepressant effects. It is a direct precursor to the neuromodulator phenethylamine, a commonly used dietary supplement. As an essential amino acid, phenylalanine is not synthesized de novo in humans and other animals, who must ingest phenylalanine or phenylalanine-containing proteins. [Provide by Wikipedia: Phenylalanine]

PRINCIPLE OF THE ASSAY

This Phenylalanine Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of Phenylalanine present in serum, urine and other 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 (λex/em = 530/585 nm) is linear to the L-phenylalanine concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Enzyme A (lyophilized)	1 vial	-20°C
Enzyme B	120 µL	-20°C
NAD Solution	1 mL	-20°C
Probe	750 µL	-20°C
Standard	120 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Deionized or distilled water
- Black flat-bottom 96 well microplate
- Pipettes, pipette tips and multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Urine: If urine samples contain visible particulates, then the samples should be centrifuged for 5 minutes at 10,000 x g at 4°C.

Tissue and cell lysate: Tissue (20 mg) or cells (2 x 10^6) can be homogenized in 200 µL of ice-cold PBS, followed by centrifugation at 10,000 x g for 5 minutes at 4°C. Use clear supernatant for assay.

Note:

- Samples not measured the same day should be stored frozen, preferably at-80°C.

REAGENT PREPARATION

- **Reconstitute Enzyme A:** add 120 µL of Assay Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store the Reconstitute Enzyme A at 4°C (**DO NOT FREEZE**) and use within 1 month.
- **Working Reagent:** for each reaction, mix 85 µL of Assay Buffer, 8 µL of NAD Solution, 5 µL of Probe, 1 µL of Reconstitute Enzyme A and 1 µL of Enzyme B.
- **Blank Working Reagent:** for each reaction, mix 86 µL of Assay Buffer, 8 µL of NAD Solution, 5 µL of Probe and 1 µL of Enzyme B (No Enzyme A).
- **Standards:** Mix 6 µL of the provided 20 mM Standard with 394 µL of distilled water to make a 300 µM Premix. Dilute standard as follows.

Standard tube	Standard (µM)	Distilled water (µL)	Standard Premix (µL)
S1	300	0	90
S2	200	30	60
S3	100	60	30
S4	0	90	0

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Briefly centrifuge tubes before opening. Keep the enzyme tube on ice during assay.

	Standard well	Sample well	Blank well
Each diluted Standard	10 µL		
Each Sample		10 µL	10 µL
Working Reagent	90 µL	90 µL	
Blank Working Reagent			90 µL
Tap plate to mix briefly and thoroughly. Incubate for **20 minutes** at **room temperature** in the dark.			
Read the fluorescence intensity at λex/em = 530/585 nm.			

CALCULATION OF RESULTS

1. Plot the L-phenylalanine standard curve and determine its slope. The Phenylalanine concentration of a Sample is calculated as follow:

   Phenylalanine (µM) = [(RFU_{SAMPLE} – RFU_{BLANK}) / Slope]

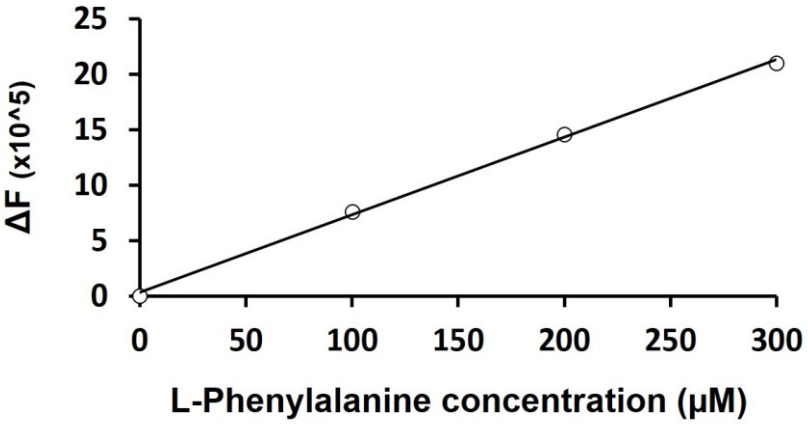
   Note:

   - RFU_{SAMPLE} and RFU_{BLANK}: the fluorescence intensity values of sample and blank, respectively.
   - Slope is the slope of the standard curve in μM^{-1}.
   - If the Sample L-phenylalanine concentration is higher than the 300 µM, dilute sample in distilled water and repeat the assay. Multiply result by the dilution factor.

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

EXAMPLE OF RESULT

The following figures demonstrate typical results with the Phenylalanine Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

2 μM