Phenylalanine Fluorometric Assay Kit

Cat. No. *AgK-437*

Lot. No. (See product label)

Product Name

Phenylalanine Fluorometric Assay Kit

Product Overview

Phenylalanine Assay Kit provides a quick, simple, accurate method for quantifying PHE in biological samples. In the assay, PHE is reductively deaminated with the simultaneous formation of NADH which reacts with our fluorescent probe to generate fluorescence at Ex/Em=535/587 nm. The assay is linear in the range from 0.1 to 1.0 nmol (2-20 µM) of Phenylalanine.

Description

L-Phenylalanine (PHE) is an electrically-neutral amino acid, one of the twenty common and one of the three aromatic amino acids used to biochemically form proteins. Phenylalanine uses the same active transport channel as tryptophan to cross the blood-brain barrier, and, in large quantities, interferes with the production of serotonin. Errors in PHE metabolism lead to phenylketonuria or PKU which can have dire consequences.

Applications

The assay is linear in the range from 0.1 to 1.0 nmol (2-20 µM) of Phenylalanine.

Size

100 assays

Kit Components

• Phenylalanine Assay Buffer
• Tyrosinase
• Enzyme Mix
• Developer
• Phenyalanine Standard (10.0 µmol)

Materials Required but Not Supplied

Tyrosinase, Enzyme Mix, Developer: Dissolve with 220 µl Assay Buffer separately. Pipette gently to dissolve. Keep on ice. Store at -20°C. Stable for at least two months
Phenylalanine Standard: Dissolve in100 µl dH2O to generate a 10mM solution. Store at -20 °C.

Detection method

Fluorescence (Ex/Em 535/587 nm)

Assay Protocol

1. Standard Curve Preparations: Dilute the Phenylalanine Standard to 0.1 mM by adding 10 µl of the Standard to 990 µl of dH2O, mix well. Add 0, 2, 4, 6, 8, 10 µl to a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol per well of the Phenylalanine Standard.
2. Sample Preparation: Tissue (20 mg) or cells (2x106) can be homogenized in 100 µl Assay Buffer, spin 15000g for 10 min to remove the insoluble material. Phenylalanine concentrations can vary over a rather wide range (normal range: 4-250 µM to over 1mM in serum in pathological states. Serum should be deproteinized either using a deproteinizing kit or a 10 kd molecular weight cut off spin filter. Take samples between 1-50 µl and adjust the well volume to 50 µl with Assay Buffer. For unknown samples, it may be necessary to test several different doses to ensure the readings are within the range of the standard curve.
3. Sample pretreatment:
The enzyme used in the assay can react with tyrosine and methionine as well as phenylalanine. Serum methionine concentrations are generally low enough to be insignificant in this assay. Tyrosine concentrations may interfere. If tyrosine interference is a concern, add 5 µl tyrosinase to the samples and preincubate for 10 minutes before performing the assay to remove tyrosine interference.
4. Development:
Mix enough reagent for the number of samples and standards to be performed. For each well, prepare a total 50

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µl Reaction Mix.
Assay Buffer 46 µl
Enzyme Mix* 2 µl
Developer 2 µl
Mix and add 50 µl of the Reaction Mix to each well containing Phenylalanine Standard or samples.
5. Incubate at 37°C for 20 minutes, protect from light.
6. Measure fluorescence (Ex/Em 535/587 nm) in a plate reader.
7. Calculation: Correct background by subtracting the 0 Phenylalanine standard from all readings (Note: The background can be significant and must be subtracted). Plot standard curve nmol/well vs. standard readings. Apply sample readings to the standard curve to get the amount of Phenylalanine in the sample wells. The Phenylalanine concentration in the test samples:
C = Ay/Sv (nmol/µl; or mM)
Where: Ay is the amount of Phenylalanine (nmol) in your sample from the standard curve.
Sv is the sample volume (µl) added to the sample well.
Phenylalanine molecular weight: 165.2 g.mol-1

Storage

Store at -20°C.

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