ab83376

Phenylalanine Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Phenylalanine levels in various samples

This product is for research use only and is not intended for diagnostic use.

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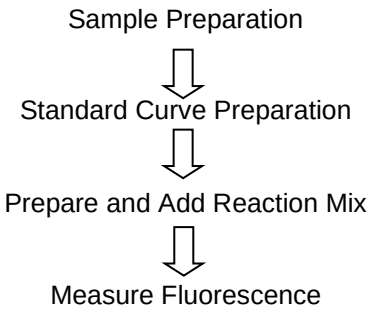
Table of Contents

1. Overview

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.

Abcam's Phenylalanine Assay Kit (Fluorometric) 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Fructose Assay Buffer	25 mL
Tyrosinase	1 vial
Phenylalanine Enzyme Mix	1 vial
Phenylalanine Developer Mix	1 vial
Phenylalanine Standard	1 vial

* Store kit at -20°C, protect from light and moisture. Warm Fructose Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

TYROSINASE, PHENYLALANINE ENZYME MIX, PHENYLALANINE DEVELOPER MIX: Dissolve with 220 µl Fructose Assay Buffer separately. Pipette gently to dissolve. Keep on ice. Store at -20°C. Stable for at least two months

PHENYLALANINE STANDARD: Dissolve in 100 µl dH_2O to generate a 10 mM solution. Store at -20°C

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorometric microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparations:

The following protocol can be proportionally scaled up for preparation of larger or smaller sample volumes.

Tissue (20 mg) or cells (2 x 106) can be homogenized in 100 µl Fructose Assay Buffer. Centrifuge at 15,000 x g for 10 minutes to remove insoluble materials. Serum should be deproteinized either using a deproteinization kit (Abcam Catalog: ab284939) or a 10 kDa molecular weight cut off spin filter (Abcam Catalog: ab93349). Add samples between 1-50 µl and adjust the well volume to 50 µl with Fructose Assay Buffer.

Notes:

a) Phenylalanine concentrations can vary over a rather wide range (normal range: 4-250 µM to over 1mM in serum in pathological states).

b) 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 2 µl tyrosinase to the samples and preincubate for 10 minutes before performing the assay to remove tyrosine interference.

For unknown samples, it may be necessary to test several different doses to ensure the readings are within the range of the standard curve

2. Standard Curve Preparation:

Dilute the Phenylalanine Standard to 0.1 mM by adding 10 µl of the Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl to a series of wells.

Adjust volume to 50 µl/well with Fructose Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol per well of the Phenylalanine Standard.

3. Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

Fructose Assay Buffer	46 µl
Phenylalanine Enzyme Mix	2 µl
Phenylalanine Developer Mix	2 µl

*** Note:** NADH and NADPH will generate background for the assay. In the absence of Phenylalanine Enzyme Mix, the assay can detect NADH and NADPH background; replace the Phenylalanine Enzyme Mix with 2 µl of Fructose Assay Buffer. The background reading can be subtracted from Phenylalanine readings.

4. Add 50 µl of Reaction Mix to each well containing the Phenylalanine Standard or samples. Incubate for 60 min at 37°C, protect from light.

5. **Measurement:** Measure fluorescence (Ex/Em 535/587 nm) in a plate reader.

5. Data Analysis

Correct background by subtracting the value of the zero Phenylalanine standard from all readings. The background reading can be significant and must be subtracted.

Plot the 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:

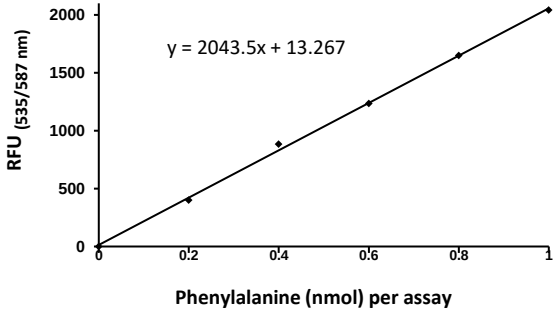
Concentration = Ay / Sv (nmol/µl; or mM)

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



Phenylalanine standard curve generated using this kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

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