BioVision

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For research use only

# Phenylalanine Fluorometric Assay Kit

(Catalog #K572-100; 100 assays; Store at -20ºC)

## I. Introduction:

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. **BioVision's 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.

## II. Kit Contents:

| Components | K572 -100 | Cap Code | Part Number |
|---|---|---|---|
| Phenylalanine Assay Buffer | 25 ml | WM | K572 -100-1 |
| Tyrosinase | lyophilized | Blue | K572 -100-2 |
| Enzyme Mix | lyophilized | Green | K572 -100-3 |
| Developer | lyophilized | Red | K572 -100-4 |
| Phenyalanine Standard (1 µmol) | lyophilized | Yellow | K572 -100-5 |

## III. Storage and Handling:

Store kit at -20ºC, protect from light and moisture. Warm up Phenylalanine Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

## IV. Reagent Preparation and Storage Conditions:

**Tyrosinase, Enzyme Mix, Developer**: Dissolve with 220 µl Assay Buffer separately. Pipette gently to dissolve. Keep on ice. Store at -20ºC. Stable for at least two months

**Phenylalanine Standard:** Dissolve in100 µl $dH_2O$ to generate a 10mM solution. Store at -20ºC.

## V. Phenylalanine 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 $dH_2O$, 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 (2 x $10^6$) 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 (BioVision # K808-200) or a 10 kd molecular weight cut off spin filter (BioVision # 1997-25). 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 min before performing the assay to remove tyrosine interference.
4. **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.

| | |
|---|---|
| Assay Buffer | 46 µl |
| Enzyme Mix* | 2 µl |
| Developer | 2 µl |

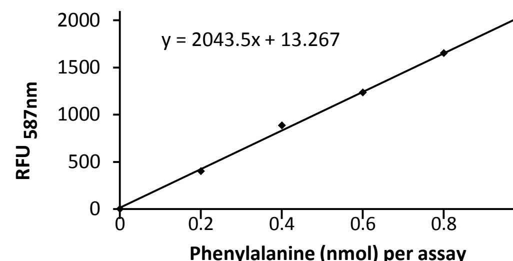
Mix and add 50 µl of the Reaction Mix to each well containing Phenylalanine Standard or samples.

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

5. Incubate at 37ºC for 60 min, 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 $g.mol^{-1}$




Phenylalanine Standard Curve generated following the kit protocol

**RELATED PRODUCTS:**

| | |
|---|---|
| Fatty Acid Assay Kit | Triglyceride Assay Kit |
| Glucose Assay Kit | Cholesterol, LDL/HDL Assay Kits |
| Glutathione Assay Kit | Ethanol and Uric Acid Assay Kit |
| NAD/NADH and NADP/NADPH Assay Kit | Lactate Assay Kits |
| TAC Total Antioxidant Capacity Kit | Pyruvate Assay Kit |
| Total Amino Acid Assay Kit | Glycogen/Starch Assay Kit |
| Urea Assay Kit | Ammonia Assay Kit |
| Coenzyme A Assay Kit | Ascorbic Acid Assay Kit |
| Glycerol Assay Kit | Alanine Assay Kit |
| ADP and ATP Assay Kits | NAD/NADH Assay Kit |

***FOR RESEARCH USE ONLY! Not to be used on humans.***

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GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of ice-cold assay buffer<br>• Omission of a step in the protocol<br>• Plate read at incorrect wavelength<br>• Use of a different 96-well plate | • Assay buffer must be at room temperature<br>• Refer and follow the data sheet precisely<br>• Check the wavelength in the data sheet and the filter settings of the instrument<br>• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type<br>• Samples prepared in a different buffer<br>• Samples were not deproteinized (if indicated in datasheet)<br>• Cell/ tissue samples were not completely homogenized<br>• Samples used after multiple free-thaw cycles<br>• Presence of interfering substance in the sample<br>• Use of old or inappropriately stored samples | • Refer data sheet for details about incompatible samples<br>• Use the assay buffer provided in the kit or refer data sheet for instructions<br>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated<br>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope<br>• Aliquot and freeze samples if needed to use multiple times<br>• Troubleshoot if needed, deproteinize samples<br>• Use fresh samples or store at correct temperatures till use |
| Lower/ Higher readings in Samples and Standards | • Improperly thawed components<br>• Use of expired kit or improperly stored reagents<br>• Allowing the reagents to sit for extended times on ice<br>• Incorrect incubation times or temperatures<br>• Incorrect volumes used | • Thaw all components completely and mix gently before use<br>• Always check the expiry date and store the components appropriately<br>• Always thaw and prepare fresh reaction mix before use<br>• Refer datasheet & verify correct incubation times and temperatures<br>• Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components<br>• Pipetting errors in the standard<br>• Pipetting errors in the reaction mix<br>• Air bubbles formed in well<br>• Standard stock is at an incorrect concentration<br>• Calculation errors<br>• Substituting reagents from older kits/ lots | • Thaw and resuspend all components before preparing the reaction mix<br>• Avoid pipetting small volumes<br>• Prepare a master reaction mix whenever possible<br>• Pipette gently against the wall of the tubes<br>• Always refer the dilutions in the data sheet<br>• Recheck calculations after referring the data sheet<br>• Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength<br>• Samples contain interfering substances<br>• Use of incompatible sample type<br>• Sample readings above/below the linear range | • Check the equipment and the filter setting<br>• Troubleshoot if it interferes with the kit<br>• Refer data sheet to check if sample is compatible with the kit or optimization is needed<br>• Concentrate/ Dilute sample so as to be in the linear range |
| **Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems. | | |