Product Manual

# Phenylalanine Assay Kit

**Catalog Number**

**MET-5195** **100 assays**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

## Introduction

Phenylalanine is an essential, non-polar amino acid that is not synthesized in humans and must be obtained through food sources such as eggs, dairy, meat and soy. It serves as precursor for tyrosine, dopamine, norepinephrine, epinephrine, and melanin. High levels of phenylalanine in the blood could result in brain damage and developmental problems. These health risks are evident in individuals who cannot metabolize phenylalanine from a genetic disorder called phenylketonuria (PKU). Individuals with PKU need to monitor the intake of phenylalanine.

Cell Biolabs' Phenylalanine Assay Kit is a simple assay for measuring phenylalanine levels in biological samples. Phenylalanine levels may be quantified in a wide range of biological samples including serum and plasma. The kit has a detection sensitivity limit of 15.6 µM phenylalanine. Each kit provides sufficient reagents to perform up to 100 assays*, including standard curve and unknown samples.

***Note: Each sample replicate requires 2 assays, one treated with L-phenylalanine dehydrogenase (+PheDH) and one without (-PheDH). Phenylalanine levels are calculated from the difference in OD readings from the 2 wells.***

## Assay Principle

The Phenylalanine Assay Kit is a sensitive, quantitative colorimetric assay for phenylalanine. The unknown samples or Phenylalanine standards are added to a 96 well plate followed by the Colorimetric Probe Mix containing WST-1, an electron mediator, and L-Phenylalanine Dehydrogenase (PheDH). During a brief incubation, WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of phenylalanine in the unknown samples is determined by comparison with a predetermined Phenylalanine standard curve.

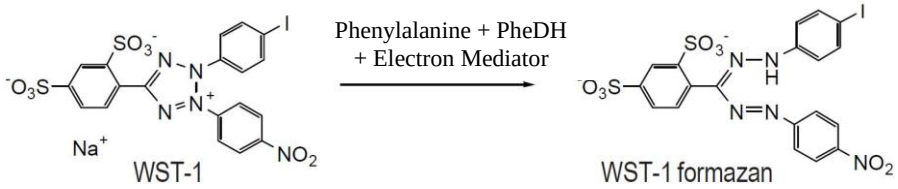


**Figure 1. Assay Principle.**

## Related Products

1. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5071: Taurine Assay Kit
4. MET-5093: Alanine Assay Kit
5. MET-5136: D-Amino Acid Kit (Colorimetric)

## Kit Components (shipped on blue ice)

1. L-Phenylalanine Standard (Part No. 51951C): One 50 µL vial at 100 mM.
2. 10X Assay Buffer (Part No. 51952A): One 25 mL bottle.

3. L-Phenylalanine Dehydrogenase (100X) (Part No. 51953D): One 200 µL vial at 5.8 U/mL

   *Note: 1 Unit corresponds to the amount of enzyme which catalyzes the formation of 1 µmol of NADH per minute at pH 10.4 and 25°C.*

4. 25X NAD+ (Part No. 51954D): One 800 µL vial.
5. 20X Colorimetric Probe (Part No. 51955C): One 1 mL amber vial.

## Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate
3. Spectrophotometric microplate reader capable of reading absorbance at 450 nm
4. 10 kD cutoff centrifugal filter units

## Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Store all remaining components at -80ºC. Avoid multiple freeze/thaw cycles. The 20X Colorimetric Probe is light sensitive and must be stored accordingly.

## Preparation of Reagents

*Note: All reagents must be brought to room temperature prior to use.*

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Prepare a Reaction Mix by diluting the 20X Colorimetric Probe, the L-Phenylalanine Dehydrogenase (100X) and the 25X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 10 assays, add 75 µL of 20X Colorimetric Probe, 15 µL of Phenylalanine Dehydrogenase (100X), and 60 µL of 25X NAD+ to 1350 µL of 1X Assay Buffer.

  *Note: Scale the described example appropriately and prepare only enough for immediate use.*

- Control Mix: Prepare a Control Mix by diluting the 20X Colorimetric Probe and the 25X NAD+ to 1X concentration in 1X Assay Buffer. For example, for 10 assays, add 75 µL of 20X Colorimetric Probe, and 60 µL of 25X NAD+ to 1365 µL of 1X Assay Buffer.

  *Note: Scale the described example appropriately and prepare only enough for immediate use.*

## Preparation of Samples

*Notes: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with unknown samples.*

- Cell lysates: Resuspend cells in 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant and filter the solution with a 10kD cutoff centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through may be assayed undiluted or diluted as necessary into 1X Assay Buffer.
- Tissue lysates: Sonicate or homogenize tissue sample in 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect the supernatant and filter the solution with a 10kD cutoff

centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through may be assayed directly or diluted as necessary into 1X Assay Buffer.

- Serum, Plasma, or Urine: Deproteinate the sample by running it through a centrifugal filter unit collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant as necessary into 1X Assay Buffer just prior to performing the assay.

## Preparation of Standard Curve

Prepare fresh Phenylalanine standards according to Table 1.

| Standard Tubes | 100 mM L-Phenylalanine Solution (µL) | 1X Assay Buffer (µL) | L-Phenylalanine (µM) |
|---|---|---|---|
| 1 | 5 | 495 | 1000 |
| 2 | 250 of Tube #1 | 250 | 500 |
| 3 | 250 of Tube #2 | 250 | 250 |
| 4 | 250 of Tube #3 | 250 | 125 |
| 5 | 250 of Tube #4 | 250 | 62.5 |
| 6 | 250 of Tube #5 | 250 | 31.3 |
| 7 | 250 of Tube #6 | 250 | 15.6 |
| 8 | 0 | 250 | 0 |

**Table 1. Preparation of Phenylalanine Standards**

## Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   *Note: Each sample replicate requires two paired wells, one to be treated with PheDH (Reaction Mix) and one without the enzyme (Control Mix) to measure endogenous sample background.*

2. Add 50 µL of each sample (Phenylalanine standard or unknown) into wells of a 96-well plate.
3. Add 150 µL of Reaction Mix to the standards and to one half of the paired sample wells and mix the well contents thoroughly.
4. Add 150 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate at room temperature for 5 minutes.

   *Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.*

6. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

## Example of Results

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

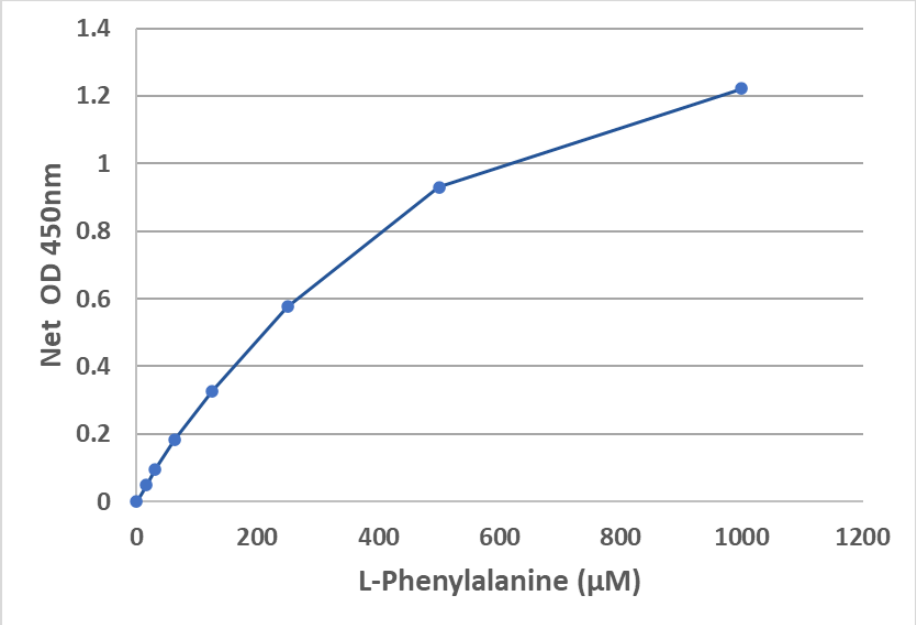


**Figure 2. Phenylalanine Standard Curve.**

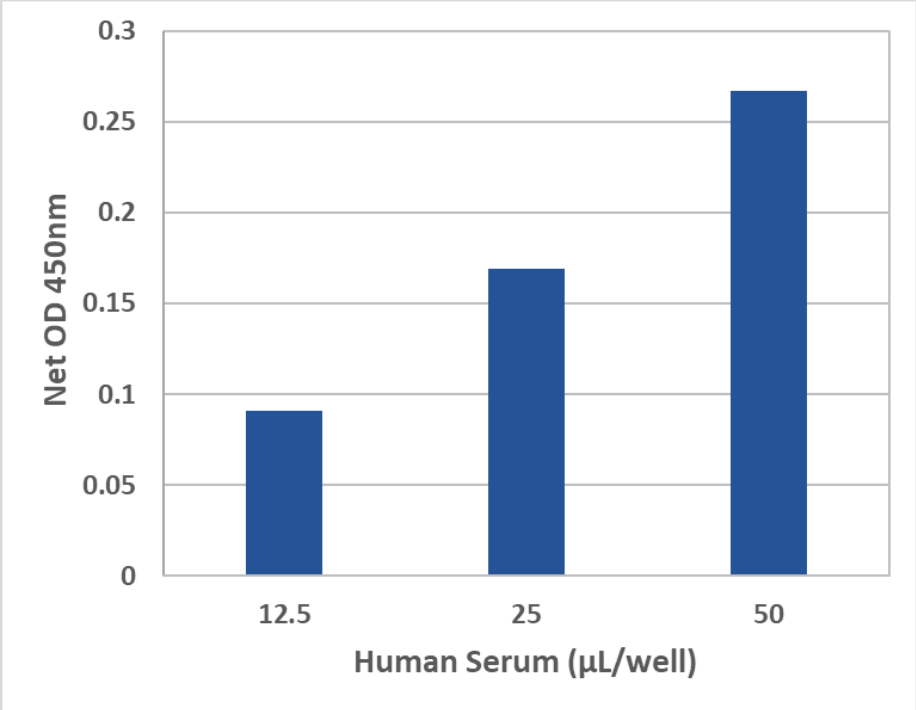


**Figure 3. Detection of Phenylalanine in Serum.** Pooled human serum was deproteinated by centrifugation in a centrifugation filter (10 kDa MWCO). The flow through was assayed according to the kit protocol.

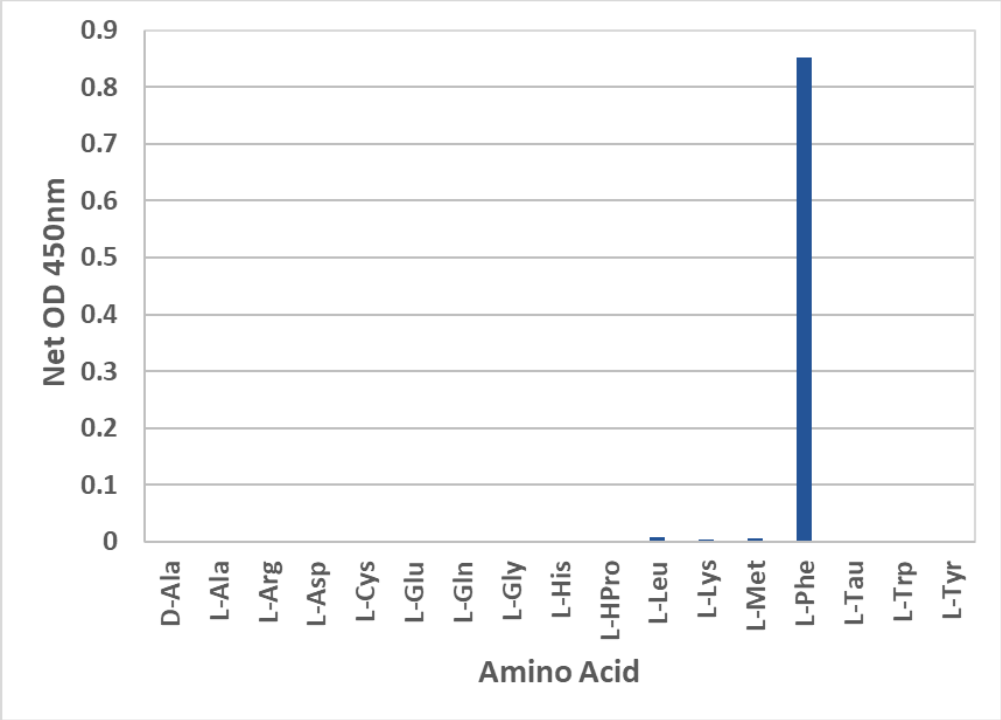


**Figure 4. Specificity of Phenylalanine Assay.** Each amino acid (500 µM) was assayed according to the kit protocol. D-alanine (D-Ala), L-alanine (L-Ala), L-arginine (L-Arg), L-asparate (L-Asp), L-cysteine (L-Cys), L-glutamate (L-Glu), L-glutamine (L-Gln), L-glycine (L-Gly), L-histidine (L-His), L-hydroxyproline (L-Hpro), L-leucine (L-Leu), L-lysine (L-Lys), L-methionine (L-Met), L-phenylalanine (L-Phe), L-taurine (L-Tau), L-tryptophan (L-Trp), L-tyrosine (L-Tyr)

## Calculation of Results.

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without PheDH (-PheDH) from the sample well values containing enzyme (+PheDH) to obtain the difference. The absorbance difference is due to the enzyme PheDH activity:

$$\Delta A = A_{(+PheDH)} - A_{(-PheDH)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of phenylalanine present in the sample. Only use values within the range of the standard curve.

## References

1. Asano Y, Akiki N, and Endo S (1987) *J. Biol Chem* **262**: 10346-10354
2. Hafid NA and Christodoulo J (2015) Transl Pediatr **4**: 304-317
3. Lopez MJ and Mohiuddin SS (2024) Biochemistry, Essential Amio Acids in StatPearls Publishing
4. Broadley KJ (2010) Pharmacol Ther **125**: 363-375

## Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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