

Version 3a, Last updated 10 June 2025

ab241000 Phenylalanine Assay Kit (Colorimetric)

For the determination of Phenylalanine concentration in biological samples such as serum or cell and tissue lysates.

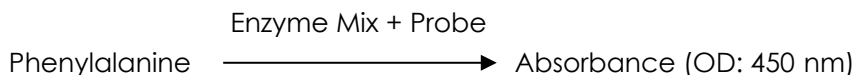
This product is for research use only and is not intended for diagnostic use.

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1. Overview

Phenylalanine Assay Kit (Colorimetric) (ab241000) provides a quick, simple and accurate method for quantifying Phenylalanine (Phe) in biological samples. In the assay, Phe is metabolized with the simultaneous formation of NADH which reacts with a probe to generate absorbance that can be followed colorimetrically at 450 nm. The assay is linear in the range from 0.2 to 10 nmol, allowing accurate determination of concentrations in the range of 1 - 50 μ M Phenylalanine.



2. Protocol Summary

Prepare tissue or cell samples, positive control.



Prepare standard curve.



Add 2 μL tyrosinase to the samples and preincubate for 10 minutes at room temperature.



Prepare reaction mix and add to standards, positive control and sample wells.



Read absorbance at 450 nm in endpoint mode.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Assay Buffer 63	25 mL	-20°C
Tyrosinase	1 vial	-20°C
Phenylalanine Enzyme Mix	1 vial	-20°C
Developer Solution III	1 vial	-20°C
Phenylalanine Standard	1 vial	-20°C

PLEASE NOTE: Assay Buffer 63 was previously labelled as Assay Buffer LXIII and Phenylalanine Assay Buffer, and Developer Solution III as Phenylalanine Developer. The composition has not changed.

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Plate reader capable of 37 °C setting and absorbance readings.
- 96-well clear plate with flat bottom.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Assay Buffer 63:

Ready to use as supplied. Store at -20°C. Warm to RT before use. Use within six months.

6.2 Tyrosinase:

Add 220 µL of Assay Buffer 63 to vial. Mix well. Store at - 20 °C. Use within six months.

6.3 Phenylalanine Enzyme Mix:

Add 220 µL of Assay Buffer 63 to vial. Mix well. Store at - 20 °C. Use within six months.

6.4 Developer Solution III:

Add 220 µL of Assay Buffer 63 to vial. Mix well. Store at - 20 °C. Use within six months.

6.5 Phenylalanine Standard:

Dissolve in 100 µL of dH₂O to generate a 10 mM solution. Store at -20°C. Use within six months.

7. Standard Preparation

– Always prepare a fresh set of standards for every use.

7.1 Prepare 1 mM Phenylalanine Standard by adding 10 μL of the 10 mM Phenylalanine Solution to 90 μL Assay Buffer 63. Mix thoroughly.

7.2 Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM Phenylalanine Standard to each well individually to generate standards of 0, 2, 4, 6, 8, and 10 nmol Phenylalanine/well. Adjust the volume of each well to 100 μL with Assay Buffer.

Standard #	Phenylalanine Standard (μL)	Assay Buffer (μL)	Phenylalanine Standard nmol/well
1	0	100	0
2	2	98	2
3	4	96	4
4	6	94	6
5	8	92	8
6	10	90	10

8. Sample Preparation

- 8.1 Tissues and cells can be homogenized in the Assay Buffer; use 100 μ L Assay Buffer for every 10 mg tissue/ 1×10^6 cells.
- 8.2 Using a dounce or other homogenizer, rapidly homogenize sample, with buffer, on ice.
- 8.3 Clarify samples by centrifugation at 10,000 $\times g$ for 5 minutes and use supernatant. Serum samples should be deproteinized using a 10 kD spin column.
- 8.4 After clarification (cells)/filtration (serum), transfer the filtrate to a fresh tube and add equal volume (5-100) μ L of each sample into two wells (A background control is needed for each volume of sample) of a 96-well clear plate.
- 8.5 Bring the volume of each well to 100 μ L with Assay Buffer 63.

9. Sample Pretreatment

- 9.1 The enzyme mix used in the assay can react with tyrosine and methionine as well as phenylalanine.
- 9.2 Serum methionine concentrations are generally low enough to not affect this assay; however, tyrosine concentrations may interfere.
- 9.3 For this reason, add 2 μ L tyrosinase to the samples and preincubate for 10 minutes at room temperature (25°C) before performing the assay. This step will remove tyrosine interference.

10. Assay Procedure

- 10.1 Mix enough reagents for the number of samples and standards to be performed.
- 10.2 For each well (standards and one for each sample), prepare 100 μ L Reaction Mix.
- 10.3 For sample background wells, prepare 100 μ L Background Control Mix:

	Reaction Mix	Background Control Mix
Assay Buffer 63	96 μ L	98 μ L
Phenylalanine Enzyme Mix	2 μ L	-
Developer Solution III	2 μ L	2 μ L

- 10.4 Add 100 μ L Reaction Mix and 100 μ L Background Control Mix to respective sample wells. Incubate plate at 37°C for 30 minutes.
- 10.5 Read absorbance at 450 nm in endpoint mode.

11. Data Analysis

- 11.1 Subtract the 0 Phenylalanine Standard reading from all standard readings, and plot the background-subtracted Phenylalanine Standards to generate the standard curve (from 0-10 nmol Phenylalanine).
- 11.2 For sample readings, subtract the reading obtained from the parallel reaction containing Background Control Mix.
- 11.3 Apply the background-subtracted values to the standard curve to calculate Phenylalanine concentration:

$$\text{Phenylalanine Concentration, } \left(\frac{\text{nmol}}{\mu\text{l}} \text{ or } \text{mM} \right) = \left(\frac{\text{Phenylalanine amount from standard curve (nmol)}}{\text{vol of sample } (\mu\text{l})} \right) \times \text{Dilution Factor } D$$

Phenylalanine molecular weight: 165.2 g/mol

12. Typical Data

Typical data provided for demonstration purposes only.

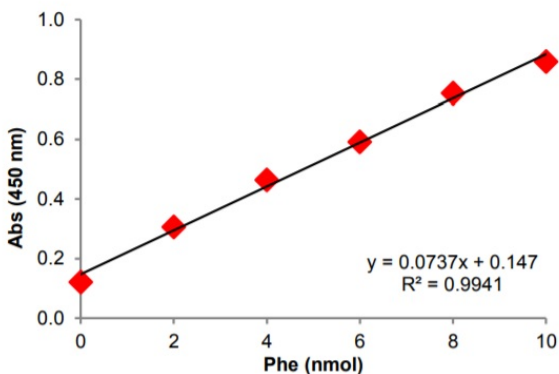


Figure 1. Phenylalanine Standard Curve: In this instance, standard curve is shown before background subtraction.

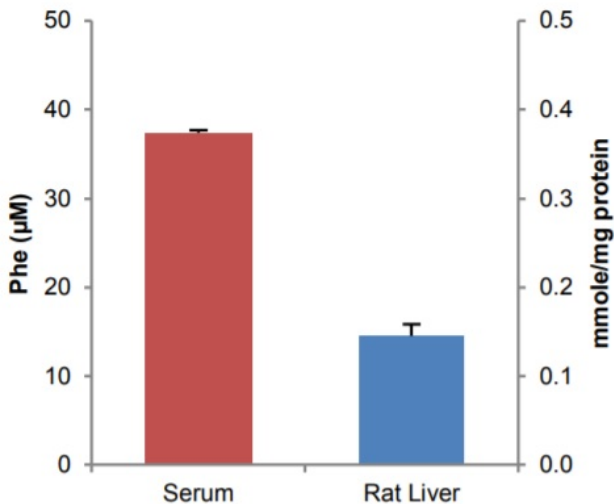


Figure 2. Determination in liver lysate and serum: Liver tissue from rat and human serum were prepared according to the protocol. For Phe determination, 10 µl liver lysate (1.5 mg/ml) and 80 µl tyrosinase-treated serum were used.

13. Notes

Technical Support

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