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Phenylalanine Assay Kit (Colorimetric)

rev 06/21

(Catalog # K481-100; 100 assays; Store at -20 °C)

I. Introduction:

L-Phenylalanine (Phe) is an electrically-neutral amino acid, one of the twenty common and one of the three aromatic amino acids building the polypeptide backbone. 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 (Colorimetric)** provides a quick, simple, accurate method for quantifying 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 µM Phenylalanine.

Phenylalanine —**Enzyme Mix + Probe**→ Absorbance (OD 450 nm)

II. Application:

- Determination of Phenylalanine concentration in biological samples e.g. serum, tissue lysates

III. Sample Types:

- Cell and Tissue Lysates
- Biological fluids: Serum

IV. Kit Contents:

Components	K481-100	Cap Code	Part Number
Phenylalanine Assay Buffer	25 ml	WM	K481-100-1
Tyrosinase	1 vial	Blue	K481-100-2
Phenylalanine Enzyme Mix	1 vial	Green	K481-100-3
Phenylalanine Developer	1 vial	Red	K481-100-4
Phenylalanine Standard (1 µmol)	1 vial	Yellow	K481-100-5

V. User Supplied Reagents & Equipment:

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

VI. Storage and Reagents Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Phenylalanine Assay Buffer:** Store at -20 °C. Warm to room temperature (RT) before use. Use within six months.
- **Tyrosinase, Phenylalanine Enzyme Mix, and Developer:** Add 220 µl of Phenylalanine Assay Buffer to each vial. Mix well. Store at -20 °C. Use within six months.
- **Phenylalanine Standard:** Dissolve in 100 µl of dH_2O to generate a 10 mM Phenylalanine Standard solution. Store at -20 °C. Use within six months.

VII. Phenylalanine Assay Protocol:

1. **Sample Preparation:** Tissues and cells can be homogenized in the Assay Buffer; use 100 µl Assay Buffer for every 10 mg tissue/1x10^6 cells. Using a Dounce Tissue Homogenizer (BV # 1998) or other homogenizer, rapidly homogenize sample, with buffer, on ice. Clarify samples by centrifugation at 10,000 x g for 5 min and use supernatant. Serum samples should be deproteinized using a 10 kDa spin column (BV #1997). After clarification (cells)/filtration (serum), transfer the filtrate to a fresh tube and add equal volume (5-100) µl of each sample into two wells (A background control is needed for each volume of sample) of a 96-well clear plate. Bring the volume of each well to 100 µl with Phenylalanine Assay Buffer.
2. **Standard Curve Preparation:** Prepare 1 mM Phenylalanine Standard by adding 10 µl of 10 mM Phenylalanine Standard solution to 90 µl Phenylalanine Assay Buffer. Mix thoroughly. 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.
3. **Sample Pretreatment:**

   The enzyme mix used in the assay can react with tyrosine and methionine as well as phenylalanine. Serum methionine concentrations are generally low enough to not affect this assay, but can be measured using BioVision's Methionine Assay Kit (K442). However, tyrosine concentrations may interfere. So, add 2 µl of tyrosinase to samples and preincubate for 10 min at RT (25 °C) before performing the assay. This step will remove the tyrosine interference.
4. **Reaction Mix:** Mix enough reagent for the number of samples and standards to be performed: For each well (standards and one for each sample), prepare 100 µl Reaction Mix. For sample background wells, prepare 100 µl Background Control Mix:

	Reaction Mix	Background Control Mix
Phenylalanine Assay Buffer	96 µl	98 µl
Phenylalanine Enzyme Mix	2 µl	-----
Phenylalanine Developer	2 µl	2 µl

Add 100 µl Reaction Mix and 100 µl Background Control Mix to respective sample wells. Incubate plate at 37 °C for 30 min.

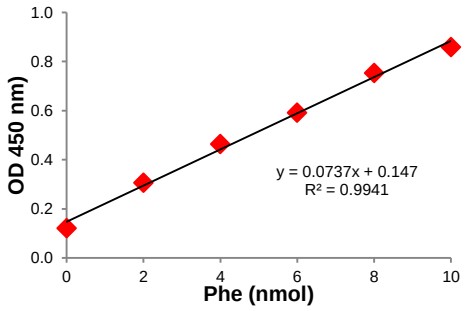
5. Measurement: Read the absorbance at 450 nm in endpoint mode.

6. Calculations: 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). For sample readings, subtract the reading obtained from the parallel reaction containing Background Control Mix. Apply the background-subtracted values to the Standard Curve to calculate Phenylalanine concentration:

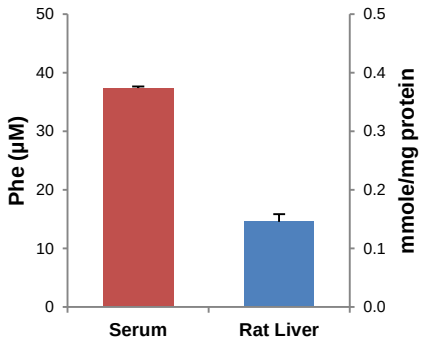
$$\textbf{Phenylalanine Concentration}, \left(\frac{nmol}{\mu l} \; or \; mM\right) = \left(\frac{\text{Phenylalanine amount from standard curve (nmol)}}{\text{vol. of sample(µl)}}\right) x \; Dilution \; Factor \; D$$

Phenylalanine molecular weight: 165.2 g/mol

a)



b)



Figures: (a) Phenylalanine Standard Curve. In this instance, Standard Curve is shown before the background subtraction **(b) Determination in liver lysate and serum.** Rat liver tissue (10 µl liver lysate (1.5 mg/ml) and human serum (80 µl tyrosinase-treated serum) were prepared according to the protocol.

VIII. Related Products:

Methionine Fluorometric Assay Kit (K442)
L-Amino Acid Quantitation Assay Kit (K639)
Glycine Assay Kit (Fluorometric) (K689)
Aspartate Colorimetric/Fluorometric Assay Kit (K552)
Glutamate Colorimetric Assay Kit (K629)
Total Polyamine Assay Kit (K475)
Alpha-Ketoglutarate Colorimetric/Fluorometric Assay Kit (K677)
Tyrosine Colorimetric Assay Kit (K573)
Phenylalanine Fluorometric Assay Kit (K572)
Cysteine Assay Kit (Fluorometric) (K558)
Alanine Colorimetric/Fluorometric Assay Kit (K652)
Diamine Oxidase Activity Assay Kit (Fluorometric) (K496)
Branched Chain Amino Acid Colorimetric Assay Kit (K564)

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