Technical Bulletin

Phenylalanine Assay Kit

Catalogue Number MAK484

Product Description

Phenylalanine is an essential amino acid and an important precursor for several key signal molecules such as dopamine, norepinephrine, and epinephrine. It is found naturally in the breast milk of mammals and used as a nutritional supplement in food and drink products. The genetic disorder phenylketonuria is the inability to metabolize phenylalanine. Individuals who cannot metabolize phenylalanine must monitor their intake of protein to control the buildup of phenylalanine.

The Phenylalanine Assay Kit uses a convenient fluorometric method to measure L-phenylalanine in biological samples. In the assay, L-phenylalanine is oxidized by phenylalanine dehydrogenase, producing NADH, which reduces a fluorescent dye to a highly fluorescent product. The resulting fluorescence intensity at λ_{Ex} = 530 nm/λ_{Em} = 585 nm is proportional to the L-phenylalanine concentration in the sample.

The linear detection range of the kit is 2 – 300 µM L-phenylalanine. The kit is suitable for L-phenylalanine activity determination in serum, urine, and other biological samples.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK484A
- Enzyme A 1 vial
  Catalogue Number MAK484B
- Enzyme B 120 µL
  Catalogue Number MAK484C
- NAD Solution 1 mL
  Catalogue Number MAK484D
- Probe 750 µL
  Catalogue Number MAK484E
- Standard (20 mM) 120 µL
  Catalogue Number MAK484F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader
- Black flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set (Catalogue Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥ 14,000 × g
- Phosphate Buffered Saline (PBS) (Catalogue Number P3813 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Enzyme A: Reconstitute vial with 120 µL of Assay Buffer. Ensure that Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at 2-8 °C (**Do not freeze**) and use within 1 month of reconstitution. Keep Enzyme A on ice during assay.

Enzyme B: Ready to use. Keep thawed enzyme on ice during assay.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Samples not assayed on the same day can be stored frozen, preferably at -70 °C.

Liquid Samples can be assayed directly.

Tissue and Cells

Homogenize tissue (20 mg) or cells (2×10^6) in 200 µL of cold 1× PBS and then centrifuge for 5 minutes at room temperature at 14,000 × g to pellet any debris. Use the clear supernatant for the assay.

All Samples

Transfer 10 µL of each Sample in duplicate, one for Sample and one for Sample Blank, to separate wells of a black 96-well plate.

Standard Curve Preparation

1. Prepare a 300 µM L-Phenylalanine Standard by mixing 6 µL of the 20 mM L-Phenylalanine Standard and 394 µL of purified water.
2. Prepare L-Phenylalanine standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1

Preparation of L-Phenylalanine Standards

Well	300 µM Standard	Purified Water	L-Phenyl-alanine (µM)
1	90 µL	0 µL	300
2	60 µL	30 µL	200
3	30 µL	60 µL	100
4	0 µL	90 µL	0

3. Mix well and transfer 10 µL of each Standard into separate wells of a black 96-well plate.

Working Reagents

1. Mix enough reagents for the number of assays to be performed.
   a. For each Sample and Standard well, prepare 100 µL of Working Reagent according to Table 2.
   b. For each Sample Blank well, prepare 100 µL of Blank Working Reagent according to Table 2.

Table 2.

Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	85 µL	86 µL
NAD Solution	8 µL	8 µL
Probe	5 µL	5 µL
Enzyme A	1 µL	-
Enzyme B	1 µL	1 µL

2. Transfer 90 µL of Working Reagent into each Standard and Sample well. Transfer 90 µL of Blank Working Reagent into each Sample Blank well. Tap plate to mix.

Measurement

1. Incubate the plate for 20 minutes at room temperature. Protect the plate from light.
2. Measure the fluorescence intensity (F) at λ_{Ex} = 530 nm/λ_{Em} = 585 nm.

Results

1. Calculate ΔF by subtracting the F reading of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔF against standard concentrations and determine the slope of the Standard curve.
3. Calculate the Phenylalanine concentration of Sample:

   L-Phenylalanine (µM) =

$$\frac{F_{Sample}-F_{Blank}}{Slope} \times DF$$

where:

F_{Sample} = Fluorescence intensity reading of Sample

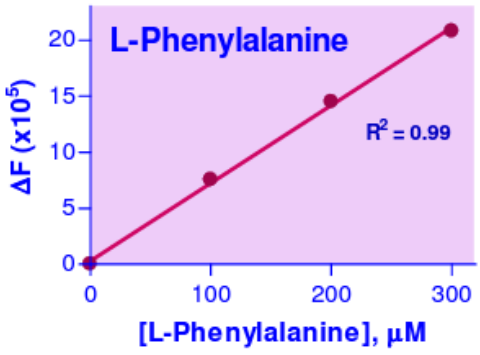
F_{Blank} = Fluorescence intensity reading of Sample Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If the Sample L-phenylalanine concentration is higher than 300 µM, dilute the sample in purified water and repeat the assay. Multiply result by the dilution factor.

Conversion factor: 1 µM L-phenylalanine is equivalent to 165 µg/L or 165 ppb.

Typical L-Phenylalanine Standard Curve



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