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Product Information

Phenylalanine Assay Kit

Catalog Number **MAK005**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Phenylalanine is a non-polar essential amino acid. In the liver, phenylalanine is converted to tyrosine, which is a precursor for multiple compounds including L-DOPA and melanin. Defects in the activity of phenylalanine hydroxylase result in the inherited metabolic disorder phenylketonuria (PKU). PKU results in the build up of phenylalanine and phenylalanine metabolites, and can result in growth defects and mental retardation if not treated.

The Phenylalanine Assay kit is suitable for L-phenylalanine detection in cell and tissue culture supernatants, serum, and other biological samples. Phenylalanine concentration is determined by a coupled enzyme assay, which results in the deamination of phenylalanine and the production of NADH which reacts with the probe resulting in a fluorescent (λ_{ex} = 535 nm/λ_{em} = 587 nm) product, proportional to the phenylalanine present.

Components

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

Phenylalanine Assay Buffer Catalog Number MAK005A	25 mL
Tyrosinase Catalog Number MAK005B	1 vl
Enzyme Mix Catalog Number MAK005C	1 vl
Developer Catalog Number MAK005D	1 vl
Phenylalanine Standard, 1 µmole Catalog Number MAK005E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Phenylalanine Assay Buffer – Allow buffer to come to room temperature before use.

Tyrosinase, Enzyme Mix, and Developer – Reconstitute each in 220 µL of Phenylalanine Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use and protect from light. Use within 2 months of reconstitution.

Phenylalanine Standard – Reconstitute in 100 µL of water to generate a 10 mM (10 nmole/µL) Phenylalanine Standard Solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Phenylalanine Standards for Fluorometric Detection
Dilute 10 µL of the 10 mM Phenylalanine Standard Solution with 990 µL of water to prepare a 0.1 mM (0.1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, 10 µL of the 0.1 mM phenylalanine standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Phenylalanine Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Serum samples should be deproteinized before use in the assay with a 10 kDa MWCO spin filter. 10–50 µL of deproteinized serum samples can be directly diluted to a final volume of 50 µL with the Phenylalanine Assay Buffer.

Tissue (20 mg) or cells (1×10^6) can be homogenized in 100 µL of the Phenylalanine Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Bring samples to a final volume of 50 µL with Phenylalanine Assay Buffer.

Notes: Samples other than serum may also be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

NADH and NADPH present in the samples may generate a background signal. To control for the background, a sample blank may be included for each sample by omitting the Enzyme Mix in the Reaction Mix.

Tyrosine present in the sample may generate a background signal. To control for tyrosine interference, the samples may be pretreated with 5 µL of Tyrosinase for 10 minutes at room temperature prior to start of the assay. Adjust the concentration of the Phenylalanine Assay Buffer in the reaction mix accordingly (see Table 1).

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Sample Blank	Samples and Standards
Phenylalanine Assay Buffer	48 µL	46 µL
Enzyme Mix	–	2 µL
Developer	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the blank, standard, and test wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 20 minutes at 37 °C. Protect the plate from light during the incubation.

3. Measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Phenylalanine standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate phenylalanine standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the sample blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of phenylalanine present in the sample may be determined from the standard curve.

Concentration of Phenylalanine

$$S_a/S_v = C$$

S_a = Amount of phenylalanine in unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of phenylalanine in sample

Phenylalanine molecular weight: 165.02 g/mole.

Sample Calculation

Amount of phenylalanine (S_a) = 5.84 nmole
Sample volume (S_v) = 50 μL
Concentration of phenylalanine in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 165.02 \text{ ng/nmole} = 19.3 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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