MA-0147

Phenylalanine Assay

(100 wells, Fluorometric, Ex/Em = 535/587 nm, Store at -20ºC)

Background:

Phenylalanine is one of the essential amino acids that we are unable to synthesize. Beyond its obvious significance as an important building block for protein synthesis, phenylalanine is also a precursor dopamine, norepinephrine, and epinephrine as well as a precursor for tyrosine which itself is a precursor to thyroid hormones. PKU is a genetic disorder characterized by the inability to degrade phenylalanine resulting in its accumulation causing neurological and cognitive impairment, at least partly due to competing for tryptophan transport channels resulting in decreased serotonin. AkrivisBio's Phenylalanine Assay is a simple, sensitive means of measuring phenylalanine in a variety of biological samples with a sensitivity in the mid to low picomole range.

Assay Principle:

Phenylalanine Dehydrogenase deaminates phenylalanine to phenylpyruvate with the formation of NADH.
NADH is used to reduce resazurin to resorufin with resultant large increase in fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0147A
Tyrosinase	lyoph	Blue	MA-0147B
Phenylalanine DH	lyoph	Green	MA-0147C
Resazurin	lyoph	Red	MA-0147D
Phenylalanine Std.	lyoph	Yellow	MA-0147E

Storage and Handling Considerations:

Store unopened assay at -20ºC. Centrifuge all vials for a few seconds before opening.
Tyrosinase: Reconstitute with 220 µl of Assay Buffer. Store at -20ºC.
Phenylalanine DH: Reconstitute with 220 µl of Assay Buffer. Store at -20ºC.
Resazurin: Reconstitute with 220 µl Assay Buffer. Store at -20ºC.
Phenylalanine Standard: Dissolve in 250 µl of DI H_2O.Store at -20ºC.

Assay Protocol:

1. Standard Curve: Add 10 µl of the 4 mM Phenylalanine Standard to 990 µl of DI H_2O, giving 40 µM standard. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl to a series of wells in a 96-well plate. Adjust all well volumed to 50 µl with Assay Buffer to giving 0, 200, 400, 600, 800, 1000 pmol of the Phenylalanine Standard.

2. Samples: Homogenize Tissue (10 mg) or cells (10^6) with 100 µl Assay Buffer. Centrifuge at 16,000 X g for 10 min to pellet particulate materials. Transfer the clear supernatant to a fresh tube. Deproteinize liquid samples (supernatant, serum, saliva, etc.) using a protein deproteinization kit (PI-0103, PI-0104) or a 10 kDa MWCO centrifugal filter (Amicon, Sartorius). Add up to 50 µl of sample to test wells. Adjust all well volumes to 50 µl with Assay Buffer.

3. Pretreatment: The Phenylalanine DH also recognizes tyrosine and methionine as substrate. Methionine concentrations are usually low enough to be ignored. Add 5 µl of tyrosinase to each sample to deplete tyrosine. Incubate for 10 minutes then proceed.
Note: The assay detects reduced pyridine nucleotides (NADH, NADPH). Previous treatment should have destroyed any present. If samples are suspected of containing significant amounts, prepare paired background control wells for each sample.

4. Initiate Reaction: Standard and test sample wells will require 50 µl of Reaction Mix. Background Control wells will require 50 µl of the control mix below. Prepare sufficient material for the total number of wells to be analyzed. The Mix contains the following (per well)

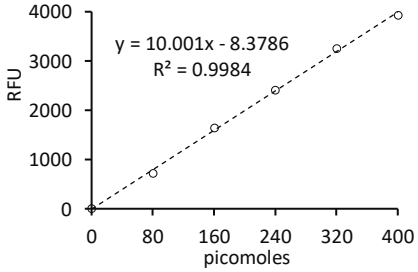
	Sample	**Background Control**
Assay Buffer	46 µl	48 µl
Phenylalanine DH	2 µl	------
Resazurin	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing Phenylalanine Standards or test samples.

5. Incubate reaction: at 37ºC for 60 minutes. You can monitor the reaction progress as an increase in fluorescence using excitation 535 nm, emission 587 nm in kinetic mode or simply wait until the reaction is complete and measure in endpoint mode.

6. Typical Results:

picomole Standard	Standard Raw Values (OD)	Background Corrected Values (OD)
0	4127.877	0
80	4844.842	716.965
160	5772.98	1645.103
240	6535.18	2407.303
320	7386.084	3258.207
400	8051.273	3923.396



7. Calculations: Subtract the zero-standard value from all other standards and test sample wells. Plot the Standard Curve and determine the slope of the standard curve. This value defines the sensitivity of the assay system. If Background Control wells have been run, subtract those values from their paired samples. Divide the background corrected sample readings by the slope of the standard curve to convert from RFU to pmoles in the wells. To extrapolate that back to the amount of phenylalanine in the original samples:

A – Divide the pmoles of phenylalanine per well by the volume of sample in µl added to the well = pmoles phenylalanine per µl of sample.
B – Multiply the pmoles phenylalanine per µl of sample by the total volume of liquid sample after deproteinization in step 2 above = total pmoles of phenylalanine per sample
C – Divide the total pmoles of phenylalanine per sample by the mg of tissue or # of cultured cells used = pmoles phenylalanine per mg tissue (or # of cells, etc.)

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