ab284571 – Phenylalanine Ammonia-Lyase Activity Assay Kit (Fluorometric)

For the measurement of Phenylalanine ammonia-lyase activity in samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284571

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
PAL Assay Buffer	40 mL	-20°C
PAL Substrate	1 vial	-20°C
Detection Reagent	1.2 mL	-20°C
PAL Positive Control	1 vial	-20°C
Ammonium Standard II	100 µL	-20°C
Microplate Sealing Film	1 Unit	-20°C

Materials Required, Not Supplied

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- 2-Mercaptoethanol
- 200-proof Ethanol
- 100% Glycerol
- 10 kDa Spin Column

Reagent Preparation

– Before using the kit, spin the tubes prior to opening. Components are stable for at least three months.

PAL Assay Buffer: Bring to room temperature (RT) before use. Store at -20°C.

PAL Substrate: Reconstitute the contents of the vial in 220 µl distilled water. Divide into aliquots and store at -20°C.

Detection Reagent: Ready to use as supplied. Divide into aliquots and store at -20°C. Keep on ice when in use.

PAL Positive Control: Reconstitute the contents of the vial in 11 µl PAL Assay Buffer and 11 µl 100% Glycerol. Divide into aliquots and store at -20°C. Keep on ice when in use. Avoid multiple freeze-thaw cycles.

Ammonium Standard II: Divide into aliquots and store at -20°C. Bring to RT before use.

Assay Protocol

Sample preparation:

1. Serum samples must be diluted at least 100-fold to avoid interference of the fluorescent signal. Serum can be diluted further, if required, with PAL Assay Buffer.
2. Add 1-50 µl of the sample into two wells labelled as Sample and Sample Background Control respectively.
3. Adjust the volume to 50 µl with PAL Assay Buffer. For Substrate Background Control, add 50 µl of PAL Assay Buffer to a well. For Positive Control, add 2 µl of PAL Positive Control into the desired well(s) and adjust the volume to 50 µl with PAL Assay Buffer.

Δ Notes:

- *For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.*
- *Samples of purified protein or recombinant enzyme must be free of ammonium sulfate or other primary amines as they will affect the fluorescent signal. If required, perform a buffer exchange using PAL Assay Buffer and 10 kDa Spin Columns Centrifuge at 12,000 x g and 4°C for 10 min and discard the filtrate. Adjust the volume of the ultra-concentrate to the original volume using PAL Assay Buffer and repeat this procedure 3-5 times. The final ultra-concentrate should be used for the PAL activity assay.*

Standard Curve Preparation

1. Dilute the 100 mM Ammonium Standard II to 1 mM Ammonium Standard II by adding 10 µl of 100 mM Ammonium Standard II to 990 µl of PAL Assay Buffer.
2. Further, dilute the 1 mM Ammonium Standard II to 200 µM Ammonium Standard II working solution by adding 200 µl of 1 mM Ammonium Standard II to 800 µl of PAL Assay Buffer.
3. Add 0, 2, 4, 6, 8 and 10 µl of 200 µM Ammonium Standard II working solution to each wells to generate 0, 0.4, 0.8, 1.2, 1.6 and 2.0 nmoles of Ammonium Standard II/well, respectively.
4. Adjust the volume of each well to 100 µl with PAL Assay Buffer.

Note: Ammonia present in the air can result in a high background

Reaction Mix Preparation

1. Mix enough reagents for the number of assays to be performed.
2. For each well, depending on the contents, either prepare a total of 50 µl Reaction Mix or 50 µl Sample Background Mix containing:

	Reaction Mix	Sample Background Mix
PAL Assay Buffer	48 µL	50 µL
PAL substrate	2 µL	-

3. Add 50 µl Reaction Mix to each well containing Sample, Substrate Background Control, and Positive Control and mix well.
4. Add 50 µl Sample Background Mix to Sample Background Control well(s) and mix well.
5. Cover the plate with a Microplate Sealing Film and incubate at 37°C for 30 min.

Developer B Preparation

While the plate is being incubated, prepare Developer B by adding 11 µl of 2-Mercaptoethanol to 1989 µL of 200-proof Ethanol. Mix well and keep on ice while in use.

Δ Note: Always prepare Developer B fresh before each experiment and keep on ice, when in use

Developer Mix preparation

1. Prepare enough Developer mix for the number of assays to be performed

	Developer Mix
PAL Assay Buffer	86 µL
Detection Reagent	7 µL
Developer B	7 µL

2. Following the 30 min incubation of the plate from the reaction mix preparation, unseal the plate and add 100 µl of Developer Mix to all wells including Sample, Sample Background Control, Substrate Background Control, Positive Control and Ammonium Standard IIs.
3. Mix well and re-seal the plate.

Measurement

Incubate the plate at 37°C for another 30 min. After the 30 min incubation, remove the Microplate Sealing Film and record the fluorescence at Ex/Em = 410/470 nm in end point mode.

Calculation

1. Subtract the 0 Standard reading from all Standard readings and Sample Background Control reading from Sample readings, respectively.
2. Plot the Standard Curve. If the Substrate Background Control is higher than the Sample Background Control, subtract the Substrate Background Control from the Sample readings instead.
3. Apply the corrected Sample readings to the Standard Curve to get the value of B nmol of Ammonium ions in the sample.

Sample Phenylalanine Ammonia-Lyase Activity = (B / Δt) X D (nmol/min) = mU

Where:

B = Amount of Ammonium ions in the sample (nmol)
Δt = Reaction time (i.e. 30 min)
D = Dilution factor (D= 1, for undiluted samples)

Unit Definition: One unit of Phenylalanine Ammonia-Lyase is the amount of enzyme that produces 1.0 µmol of Ammonium ions per minute at pH 8.0 at 37°C.

Technical Support

For all technical or commercial enquiries please go to:

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