Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

BioVision
BioVision Incorporated

Phenylalanine Ammonia-Lyase Activity Assay Kit (Fluorometric)

08/20

(Catalog # K2055-100; 100 assays; Store at -20°C)

I. Introduction:

Phenylalanine Ammonia-Lyase (PAL, EC 4.3.1.24) catalyzes the reversible non-oxidative deamination of L-phenylalanine to trans-cinnamic acid and ammonia without any external cofactors. PAL is expressed in plants, yeast and some fungi and is absent in bacteria and animals. Patients suffering from the genetic disorder, phenylketonuria is unable to metabolize phenylalanine that leads to irreversible cognitive impairment. Historically, phenylketonuria has been treated with a phenylalanine-restricted diet taken for the entirety of the patient's life. Recently, a drug containing PEGylated PAL has been FDA-approved to treat phenylketonuria, limiting the need of a restricted diet. **BioVision's Phenylalanine Ammonia-Lyase Activity Assay Kit** measures the activity of PAL via a two-step plate-based assay. The products of the PAL enzymatic reaction, generated in the first step, react with the developers resulting in the formation of a fluorescent compound, which is detected at Ex/Em = 410/470 nm. The kit can detect as low as 3 µU (3 pmol substrate catalysis/minute) of PAL under assay conditions.

L-Phenylalanine —(**Phenylalanine Ammonia-Lyase**)→ *trans*-Cinnamic acid + NH_3 —(**Developers A and B**)→ **Fluorescent Product** (Ex/Em = 410/470 nm)

II. Application:

Measurement of Phenylalanine ammonia-lyase activity in samples

III. Sample Types:

- Serum
- Purified protein or recombinant enzyme (**must be free of primary amines including ammonium sulfate**)

IV. Kit Contents:

Components	K2055-100	Cap Code	Part Number
PAL Assay Buffer	40 ml	NM	K2055-100-1
PAL Substrate	1 vial	Green	K2055-100-2
Developer A	1.2 ml	Amber	K2055-100-3
PAL Positive Control	1 vial	Blue	K2055-100-4
Ammonium Chloride Standard	100 µl	Yellow	K2055-100-5
Microplate Sealing Film	1	--	K2055-100-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- 2-Mercaptoethanol
- 200-proof Ethanol
- 100% Glycerol
- 10 kDa Spin Column (BioVision Cat# 1997)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

- **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.
- **Developer A:** 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 Chloride Standard (100 mM):** Divide into aliquots and store at -20°C. Bring to RT before use.

VII. Phenylalanine Ammonia-Lyase Activity Assay Protocol:

1. Sample Preparation: 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. Add 1-50 µl of the sample into two wells labeled as **Sample** and **Sample Background Control** respectively. 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.

Notes:

a) For Unknown Samples, we suggest testing several dilutions to ensure that the readings are within the Standard Curve range.

b) 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 (BioVision Cat# 1997). 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.

Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

BioVision
BioVision Incorporated

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

3. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. 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	-

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

4. 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.

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

5. Developer Mix Preparation: Prepare enough Developer Mix for the number of assays to be performed.

	Developer Mix
PAL Assay Buffer	86 µl
Developer A	7 µl
Developer B	7 µl

Following the 30 min incubation of the plate from step 3, 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 Chloride Standards. Mix well and re-seal the plate.

6. **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.

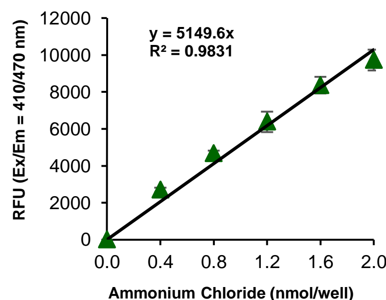
7. Calculation: Subtract the 0 Standard reading from all Standard readings and Sample Background Control reading from Sample readings, respectively. Plot the Ammonium Chloride Standard Curve. If the Substrate Background Control is higher than the Sample Background Control, subtract the Substrate Background Control from the Sample readings instead. Apply the corrected Sample readings to the Ammonium Chloride 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

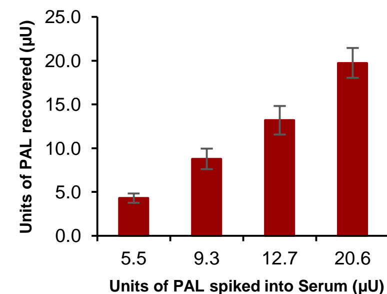
Where, **B** = Amount of Ammonium ions in the sample (nmol)
Δt = Reaction time (i.e. **30 min)**
D = Dilution factor (D= 1, for undiluted samples)

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.

a)



b)



Figures. (a). Ammonium Chloride Standard Curve. **(b).** Pooled normal human male AB serum was spiked with 5.5, 9.3, 12.7 and 20.6 µU of PAL respectively. The corresponding units of PAL recovered were 4.3, 8.8, 13.2 and 19.7 µU (recovery rates ranging from 78.4-103.7%). The data shown is the average of three replicates where experiments were performed according to the kit protocol.

VIII. Related Products:

Phenylalanine Assay Kit (Colorimetric) (K481)
Phenylalanine Fluorometric Assay Kit (K572)
Potassium (Serum) Detection Assay Kit (Fluorometric) (K940)

FOR RESEARCH USE ONLY! Not to be used on humans.