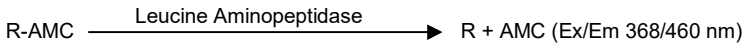
Leucine Aminopeptidase (LAP) Activity Assay Kit (Fluorometric) (BN00765)

(Catalog # BN00765; 100 assays; Store at -20°C)

I. Introduction:

Leucine aminopeptidases (EC 3.4.11.1) (LAPs) are a diverse set of exopeptidases that catalyze the hydrolysis of leucine residues from the amino-termini of proteins or peptides. LAPs are ubiquitous enzymes present among animals, plants and prokaryotes. Previously, they were thought to typically play important roles in cell maintenance, growth and development. However, research in the recent years has identified multiple secondary functions for these enzymes in animals and microbes including transcriptional regulation and vesicle transport. Studies have implicated LAP enzymes in tumor cell proliferation, invasion and angiogenesis. Placental LAP is used as a biomarker in ovarian epithelial cancer while adipocyte-derived LAP is used as a marker of endometrial cancer cell proliferation and differentiation. LAP enzymes are also known to be involved in catabolism of oxytocin and vasopressin and insulin regulation of GLUT4 receptors in diabetes. Assay Genie's Leucine Aminopeptidase assay kit provides a quick, sensitive and easy way for measuring total LAP activity in various samples. In this assay, LAPs hydrolyze leucine from the fluorescent probe and the amount of fluorescent probe detected at Ex/Em 368/460 nm is used to determine the total activity of the LAP enzymes. The assay is simple to perform, high-throughput adaptable and can detect less than 0.1 mU of LAP activity.



II. Applications:

- Measurement of leucine aminopeptidase activity in various tissues/cells.

III. Sample Type:

- Animal tissues: liver, spleen, placenta, etc.
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	BN00765	Cap Code	Part Number
LAP Assay Buffer	25 ml	WM	BN00765-1
LAP Substrate	1 vial	Blue	BN00765-2
AMC Standard (1 mM)	100 µl	Yellow	BN00765-3
LAP Positive Control	1 vial	Violet	BN00765-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom. Black plate can also be used for this assay.
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **LAP Assay Buffer:** Warm to room temperature before use. Store at either 4ºC or -20ºC.
- **LAP Substrate:** Reconstitute with 110 µl anhydrous DMSO. Aliquot and store at -20°C. Use within two months.
- **LAP Positive Control:** Reconstitute with 11 µl of dH_2O and mix thoroughly. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.
- **AMC Standard:** Warm to room temperature and mix well before use. Aliquot if required and store at –20°C. Use within two months.

VII. Leucine Aminopeptidase Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold LAP Assay Buffer and keep on ice for 10 min. Centrifuge at 10,000 x g at 4 ºC for 15 minutes and transfer the supernatant to a fresh tube. Add 5-50 µl sample per well in a 96 clear well plate & adjust the volume to 90 µl with LAP Assay Buffer. For positive control: dilute the required amount of LAP Positive Control 10 times with LAP Assay Buffer. Add 10 µl of the diluted Positive Control per well into the desired well(s) and adjust the volume to 90 µl with LAP Assay Buffer. For the **No Enzyme** control: add 90 µl of Assay Buffer to duplicate wells.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples exhibiting significant background, prepare parallel sample well(s) as background controls.

2. **AMC Standard Curve:** Dilute the 1 mM AMC standard solution 20 times by adding 10 µl of the standard solution to 190 µl Assay buffer to obtain a 0.05 mM standard solution. Add 0, 2, 4, 6, 8 and 10 µl of 0.05 mM AMC Standard into a series of wells in a clear 96 well plate to generate 0, 0.1, 0.2, 0.3, 0.4 and 0.5 nmol/well of AMC Standard. Adjust the volume to 100 µl/well with LAP Assay Buffer.

3. **Substrate Mix:** Dilute the substrate stock solution 10 times with dH_2O to obtain a 1X working solution. Make enough reagent and add 10 µl per well.

   Note: Do not add substrate to wells containing the standards.

4. **Measurement:** Measure fluorescence immediately (Ex/Em= 368/460 nm) in kinetic mode for 45-60 min. at 37°C.

   Note: Measurement time for the linear phase of the reaction depends on the LAP activity in samples. We recommend measuring the fluorescence in kinetic mode and choosing two time points (t_1 and t_2) in the linear range to calculate the LAP activity of the samples. The AMC Standard Curve can be read in endpoint mode.

5. Calculation: Subtract the 0 nmol Standard reading from all Standard Curve readings. Plot the fluorescence Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. **If sample background control reading is smaller than the No Enzyme control reading, subtract the No Enzyme control reading from the sample reading.** For all sample wells, quantify the specific fluorescence (C_S) by subtracting the fluorescence intensity of the background control (F_{BC}) from the fluorescence intensity of the sample (F_S): $C_S = F_S - F_{BC}$. LAP enzymatic activity is obtained by applying the C_S values to the AMC standard curve to get *B* nmole of the LAP substrate metabolized by LAP enzyme during the reaction time ($\Delta t = t_2 - t_1$).

Sample Leucine aminopeptidase Activity = B/(Δt X V) x D = nmol/min/ml = mU/ml

Where: **B** = AMC amount from Standard Curve (nmol)
Δt = reaction time (min)
V = sample volume added into the reaction well (ml)
D = Dilution Factor

Unit Definition: One unit of **Leucine aminopeptidase** is the amount of enzyme that generates 1.0 µmole of AMC per min at pH 8 at 37°C.

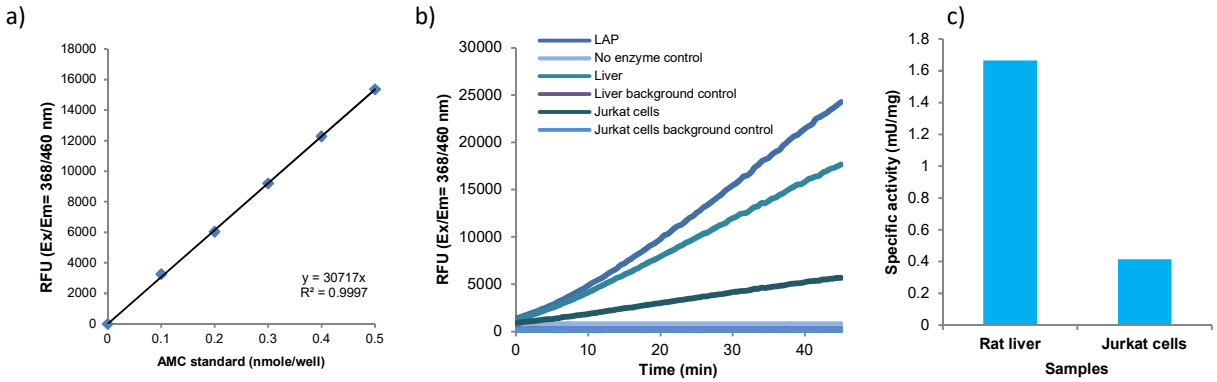


Figure: (a) AMC standard curve; (b) Reaction kinetics of leucine aminopeptidase activity in rat liver (6.6 µg protein) and Jurkat cells (8 µg protein) using appropriate background controls; (c) Leucine aminopeptidase specific activity was calculated in rat liver and Jurkat cell lysates. Assays were performed following the kit protocol.

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