ab234627 – Leucine Aminopeptidase (LAP) Activity Assay Kit (Fluorometric)

For the measuring total LAP activity in various samples such as animal tissues or cell cultures.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab234627

Storage and Stability

- Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted. Avoid repeated freeze-thaws of reagents.
- Reconstituted LAP Positive Control and LAP Substrate are stable for 2 months.
- Aliquot components in working volumes before storing at the recommended temperature.

Materials Supplied

Item	Quantity	Storage Condition
AMC Standard/AMC Standard (1 mM)	100 µl	4°C or -20°C
LAP Assay Buffer	25 ml	-20°C
LAP Positive Control	1 vial	-20°C
LAP Substrate	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

LAP Assay Buffer: Warm to room temperature before use. Store at either 4°C or -20°C.

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 dH2O 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.

Leucine Aminopeptidase Assay Protocol

Sample Preparation

1. Rapidly homogenize tissue (10 mg) or cells (1 x 106) with 100 µl ice cold LAP Assay Buffer and keep on ice for 10 min.
2. Centrifuge at 10,000 x g at 4 °C for 15 minutes and transfer the supernatant to a fresh tube.
3. Add 5-50 µl sample per well in a 96 black well plate and adjust the volume to 90 µl with LAP Assay Buffer.
4. **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.
5. **For the No Enzyme control:** add 90 µl of Assay Buffer to duplicate wells.

   Δ **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.

AMC Standard Curve:

1. 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.
2. Add 0, 2, 4, 6, 8 and 10 µl of 0.05 mM AMC Standard into a series of wells in a black 96 well plate to generate 0, 0.1, 0.2, 0.3, 0.4 and 0.5 nmol/well of AMC Standard.
3. Adjust the volume to 100 µl/well with LAP Assay Buffer.

Substrate Mix:

1. Dilute the substrate stock solution 10 times with dH2O to obtain a 1X working solution.
2. Make enough reagent and add 10 µl per well.

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

Measurement

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

  Δ **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.

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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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