QuantiChrom™ Leucine Aminopeptidase Assay Kit (DLAP-100)

Quantitative Colorimetric Assay for Leucine Aminopeptidase Activity

DESCRIPTION

LEUCINE AMINOPEPTIDASE (LAP) (E.C 3.4.11.1) is an enzyme commonly found in liver cells and intestinal cells that hydrolyzes leucine from the N-terminus of proteins and polypeptides. The expression of LAP increases in damage and immune response, so increased LAP activity in serum samples can indicate potential liver damage, tumorigenesis, or inflammation.

BioAssay Systems' LAP activity assay provides a convenient colorimetric method to measure LAP activity in biological samples. In this assay, LAP hydrolyzes a dye-linked leucine substrate, resulting in a yellow, colored product, measurable at OD405nm. The increase in absorbance at 405 nm (ΔOD) in 20 minutes is directly proportional to the LAP activity.

KEY FEATURES

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 0.025 to 250 U/L.

Fast and convenient. The procedure involves addition of a single working reagent and incubation for 20 min. The assay can be run at room temperature or 37°C.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of LAP enzyme activity in biological samples, e.g. cells, tissues, and serum samples.

For high-throughput screening of LAP inhibitors and evaluation of drug modulators.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 20 mL **Substrate:** 100 µL
Standard: 50 µL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Tissue: Prior to homogenization, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

For unknown samples, it is prudent to test several dilutions to determine an optimal sample dilution factor *n*.

Procedure using 96-well plate

1. *Standard*. Prepare 250 µL 4mM Premix by mixing 10 µL of the provided 100 mM Standard and 240 µL dH_2O. Dilute Standard in 0.5-mL centrifuge tubes as described in the Table. Transfer 10 µL Standards into separate wells of a clear flat bottom 96-well plate. Unused standards can be frozen for future use.

No	Premix + dH_2O	Standard (mM)
1	100 µL + 0 µL	4.0
2	60 µL + 40 µL	2.4
3	30 µL + 70 µL	1.2
4	0 µL + 100 µL	0

Add 190 µL Assay Buffer to Standard wells.

2. Transfer 10 µL of each sample into separate wells.
3. Prepare enough Working Reagent (WR) for all Sample wells by mixing, for each well, 1 µL Substrate Solution, and 195 µL Assay Buffer. Fresh preparation of the WR is recommended.
4. Add 190 µL WR to each sample well. Tap plate briefly but thoroughly to mix.
5. Immediately read OD_{405nm} at 0 minutes and again at 20 minutes.

CALCULATION

Subtract blank value (water, #4) from the Standard values and plot the ΔOD against their concentrations. Determine the slope and calculate the LAP activity of Sample as follows:

$$\text{LAP Activity} = \frac{OD_{20} - OD_0}{\text{Slope (mM}^{-1}) \cdot t \text{ (min)}} \times 1000 \times n \quad \text{(U/L)}$$

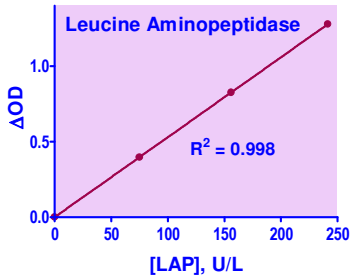
where OD_{20} and OD_0 are OD_{405nm} values of the Sample at t = 20 min and 0 min, respectively. n is the sample dilution factor.

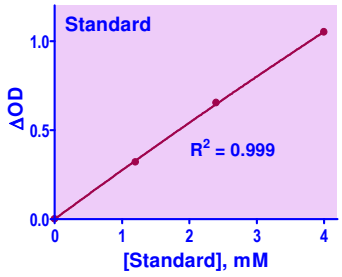
Note: if the calculated concentration is higher than 50 U/L, dilute sample in Assay Buffer and repeat the assay. Multiple the result by the dilution factor.

Unit definition: one unit of enzyme catalyzes the production of 1 µmole of product per minute under the assay conditions (pH 8.0 and 37°C).

MATERIALS REQUIRED, BUT NOT PROVIDED

LAP enzyme (e.g Sigma, Cat# L6007), pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.





EXAMPLES

Biological samples were assayed in triplicate using the 96-well protocol. The LAP activity was 2.50 ± 0.37 U/L in HEPG2, 2.18 ± 0.71 U/L in MDA-MB-231, and 1.33 ± 0.40 U/L in HeLa cell lysates. In serum, the LAP activity was 0.61 ± 0.09 U/L in bovine serum, 1.28 ± 0.12 U/L in human serum, 2.09 ± 0.93 U/L in rat serum.

LITERATURE

1. Grembecka J, Kafarski P. (2001). Leucine aminopeptidase as a target for inhibitor design. *Mini Rev Med Chem*; 1(2):133-44.
2. Hitzerd SM, Verbrugge SE, Ossenkoppele G, Jansen G, Peters GJ. (2014). Positioning of aminopeptidase inhibitors in next generation cancer therapy. *Amino Acids*; 46(4): 793-808.
3. Sato, Y. (2003). Aminopeptidases and angiogenesis. *Endothelium*; 10(6): 287-90.