Leucine Aminopeptidase

Microplate Assay Kit

User Manual

Catalog # CAK1304

(Version 1.1A)

Detection and Quantification of Leucine Aminopeptidase(LAP)Activityin Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

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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.

Leucine Aminopeptidase Microplate Assay Kit is a sensitive assay for determining Leucine Aminopeptidase activity in various samples. In this assay, LAPhydrolyze substrate and releases pNP which can be measured at absorbance. The intensity of the product color, measured at 405 nm, is proportional to the LAP activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
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Note:

Substrate:add 9 mlReaction Buffer, heat at 50-60°Cto dissolve before use; store at -20 °C for a month after reconstitution.

Standard:add 1 ml distilled water to dissolve before use, mix; then add 0.03 ml into 0.97 ml distilled water, the concentration will be 300μmol/L; store at -20 °C for a month after reconstitution.Perform 2-fold serial dilutions with distilled water.

Positive Control:add 0.5 ml Assay Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquidsamples

Liquid samples can be used directly.

V. ASSAY PROCEDURE

Warm all reagents to 37°C before use.

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Substrate	90 µl	90 µl	--	--	90 µl
Sample	10 µl	--	--	--	--
Assay Buffer	--	10 µl	--	--	--
Standard	--	--	100 µl	--	--
Positive Control	--	--	--	--	10 µl
Distilled water	--	--	--	100 µl	--
Mix, put it in the oven, incubate at 37 °C for 5 minutes.					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, measured at 405 nm and record the absorbance.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of LAP activity is the amount of enzyme that generates 1 μmol of pNP per min at 37°C.

1. According to the protein concentration of sample

$$LAP\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$LAP\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$LAP\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 300μmol/L = 0.3μmol/ml;

$V_{Standard}$:the volume of standard, 0.1 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

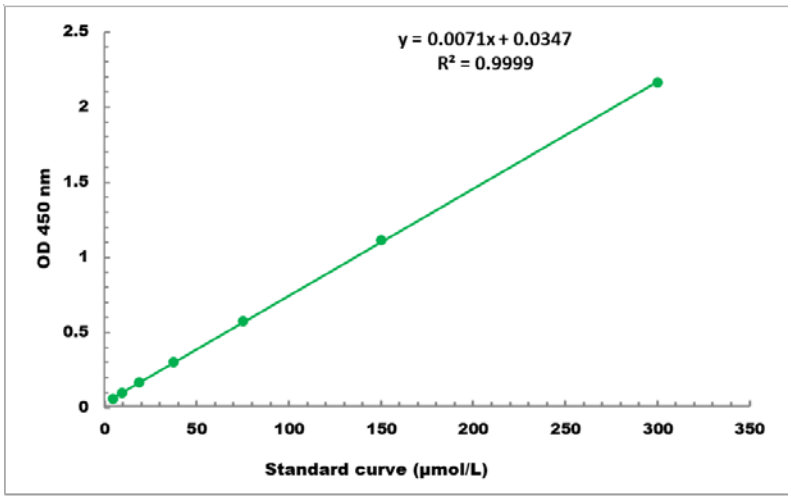
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay Buffer, 1 ml;

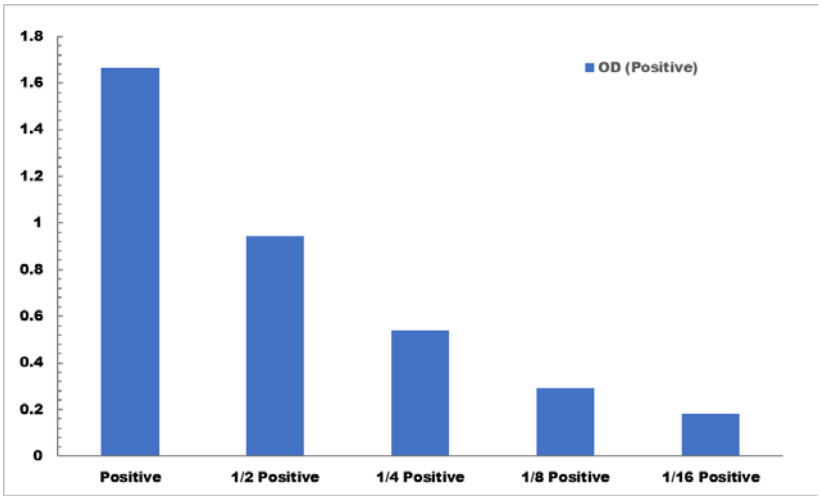
T: the reaction time, 5 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 3μmol/L - 300μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES