LAP Activity Assay Kit

ARG83562 LAP Activity Assay Kit can be used to measure LAP in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83562

Package: 100 tests

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PRINCIPLE OF THE ASSAY

ARG83562 LAP Activity Assay Kit is a sensitive assay for determining Leucine Aminopeptidase activity in various samples. In this assay, LAP hydrolyze 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Positive Control at-20 °C, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4 °C
Positive Control	1 vial (lyophilized)	-20 °C
Assay Buffer	4 x 30 ml	4 °C
Substrate	1 vial (lyophilized)	4 °C
Reaction Buffer	10 ml	4 °C
Dye Reagent	10 ml	4 °C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Positive Control at-20 °C, all other component at 2-8°C. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate with 1 ml Assay buffer per 5×10^6 cell or bacteria. Centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate:** Reconstitute the **Substrate** with **9 ml** of Reaction Buffer, heat at 50-60 °C to dissolve before use. Make sure the **Substrate** is dissolved completely and mixed thoroughly before use.

- **Positive Control:** Reconstitute the **Positive Control** with **0.5 ml** of Assay Buffer. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use.

- **Standard:** Reconstitute the **Standard** with 0.5 ml of distilled water. Allow the **Standard** keep on bench for few minutes, to yield **standard stock**. Make sure the **Standard** is dissolved completely, then add 30 µl **standard stock** into 970 µl distilled water, to yield 300 nmol/mL standard. Perform 2-fold serial dilutions of the top standards to make the standard curve.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

Summary of LAP Activity Assay Kit Procedure

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 well Incubate for 5 min at 37 °C					
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Read the OD at 405 nm					

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition: One unit of LAP activity is defined as the enzyme reduce 1 µmol NADH per minute.

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

b.) Calculation:

Formula:

a). According to the protein concentration

LAP (U/mg) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein}) \times T]$

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

b). According to the weight

LAP (U/g) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times W / V_{Assay}) \times T]$

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

c). According to the volume

LAP (U/ml) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T]$

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