

APA867Ra61 100µg
Active Phospholipase A2 Group VII (LpPLA2)
Organism Species: *Rattus norvegicus* (Rat)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Leu22~Asn440

Tags: N-terminal His-tag

Purity: >95%

Traits: Freeze-dried powder

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 5% trehalose.

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 7.6

Predicted Molecular Mass: 48.6kDa

Accurate Molecular Mass: 60kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 10mM PBS (pH7.6) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

```
LYWQDPSFFDFRPSVMFHKLQSVMSAVSAGRCKIPKNGSYYPVGCTDMMFGYGNESIFLRLYYPAQDQGPHTVWVWNIEYFWGLSKFL  
GPTTFVGNILRLLYGSLTAPASWNFPLRTGEKYPLIIFSHGLGAFRTIYSAIGAALASYGFIVATVEHRDGSASATYYFEDQAAAKMEN  
RSWFLKIKQEESERARKEQVRQRAKECSKALSAILDIEHGNPKENVLGLPFDMKQLKDSIDETKIAVMGHSFGGATVFAQLSEDRF  
RCGIALDPWMFVSEELYSRVPQLFFINSAEFQTPKDIAMKNFYQPKERKMITIKGSVHQNFDGTFVTGKIIGNKLSLKGIDISR  
VAIDLTKASLAFLQKHLGLHKDFDQWDCLEGENENLIPGSPFDVVTQSPALQSSPGSHNQN
```

[ACTIVITY]

PLA2G-VII which is also known as Lp-PLA2, is a plasma enzyme bound to lipoproteins: 80% bound to LDL, 15%-20% to HDL, and the remainder to VLDL. It is produced in major by mature macrophages and activated platelets. In contrast to other classical sPLA2s, PLA2G-VII has poor specificity toward Sn-2 long chain fatty acids, unless heavily oxidized, and undergoes the catalysis of its substrates in the aqueous phase rather than at the interfacial surface of lipids. Thus, it has high specificity for water-soluble phospholipids in plasma including oxidatively-modified phospholipids and platelet-activating factor (PAF). Because of the latter activity, it is also known as PAF acetylhydrolase (PAF-AH). Lack of human PLA2G-VII is related to a higher risk for stroke and heart disease. The activity of recombinant rat PLA2G-VII was measured by its ability to cleave a colorimetric peptide substrate

1-O-hexadecyl-2-deoxy-2-thio-S-acetyl-sn-glycerol-3-phosphorylcholine (2-Thio-PAF). The reaction was performed in 50 mM MES, 150 mM NaCl, 0.1 mg/mL BSA, pH 6.5 (Assay Buffer), initiated by addition 50 μ L of various concentrations of PLA2G-VII (diluted by Assay Buffer) to 50 μ L of 0.2 mM Substrate and 0.2 mM DTNB mixture. The final well serves as a negative control with no PLA2G-VII, replaced with 50 μ L assay buffer. Then read absorbance at 405 nm in kinetic mode for 5 minutes. The specific activity of recombinant rat PLA2G-VII is 112.37 pmol/min/ μ g.

Specific Activity (pmol/min/ μ g)=

$$\frac{\text{Adjusted } V_{\max}^* (\text{OD}/\text{min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} (\text{M}^{-1}\text{cm}^{-1}) \times \text{path corr.}^{***} (\text{cm}) \times \text{amount of enzyme (}\mu\text{g)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 $\text{M}^{-1}\text{cm}^{-1}$

***Using the path correction 0.320 cm

[IDENTIFICATION]

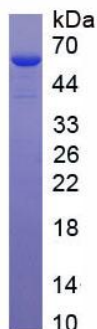


Figure 1. SDS-PAGE

Sample: Active recombinant LpPLA2, Rat

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.