

Anti-Phospholipase A2 activating protein (PLAP) Antibody [JE53-81]

ET7111-06



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size 87 kDa
Clone number:	JE53-81

Description: Plays a role in protein ubiquitination, sorting and degradation through its association with VCP. Involved in ubiquitin-mediated membrane proteins trafficking to late endosomes in an ESCRT-dependent manner, and hence plays a role in synaptic vesicle recycling (By similarity). May play a role in macroautophagy, regulating for instance the clearance of damaged lysosomes. Plays a role in cerebellar Purkinje cell development (By similarity). Positively regulates cytosolic and calcium-independent phospholipase A2 activities in a tumor necrosis factor alpha (TNF-alpha)- or lipopolysaccharide (LPS)-dependent manner, and hence prostaglandin E2 biosynthesis.

Immunogen: Synthetic peptide within N terminal Human PLAP.

Positive control: HeLa cell lysate, MCF7 cell lysate, A549 cell lysate, A549, NIH/3T3, human kidney tissue, mouse kidney tissue, rat kidney tissue, human prostate carcinoma tissue, human uterus tissue.

Subcellular location: Nucleus, cytoplasm, synapse.

Database links: SwissProt: Q9Y263 Human | P27612 Mouse | P54319 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:100
IHC-P	1:50-1:200
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 1% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

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Images

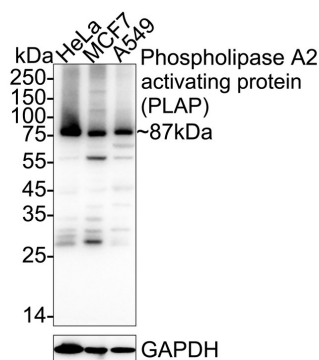


Fig1: Western blot analysis of Phospholipase A2 activating protein (PLAP) on different lysates with Rat anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/5,000 dilution.

Lane 1: HeLa cell lysate

Lane 2: MCF7 cell lysate

Lane 3: A549 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 87 kDa

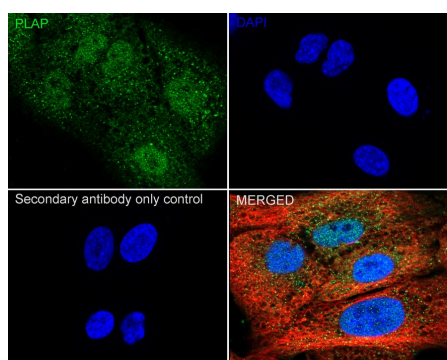
Observed band size: 87 kDa

Exposure time: 20 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7111-06) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4 °C overnight. Goat Anti-Rat IgG H&L - HRP Secondary Antibody (HA1023) at 1/5,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of A549 cells labeling Phospholipase A2 activating protein (PLAP) with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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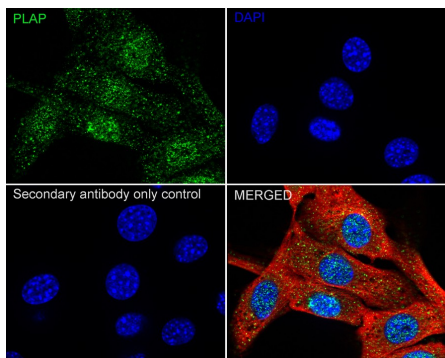
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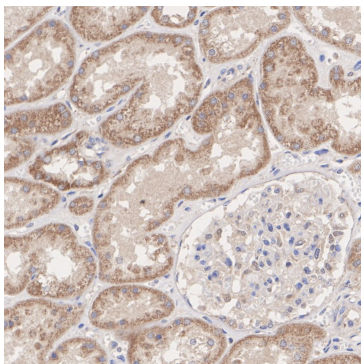
Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling Phospholipase A2 activating protein (PLAP) with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/50 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/50 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

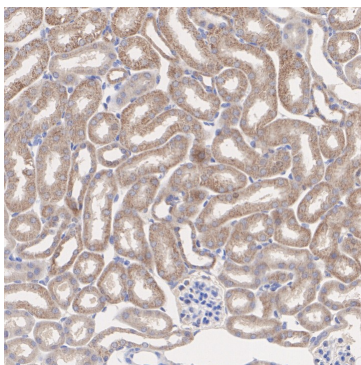
Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/200 dilution.



The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7111-06) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig5: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/200 dilution.



The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7111-06) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

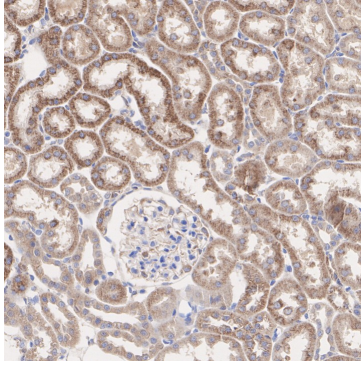


Fig6: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7111-06) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

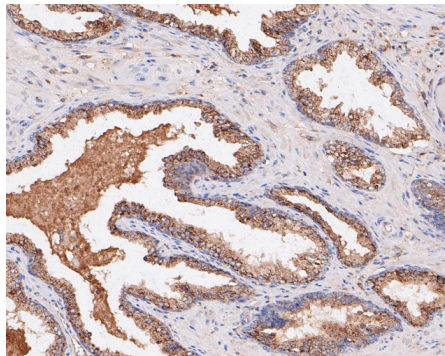


Fig7: Immunohistochemical analysis of paraffin-embedded human prostate carcinoma tissue with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7111-06) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

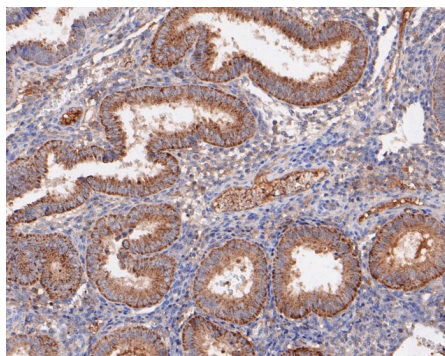


Fig8: Immunohistochemical analysis of paraffin-embedded human uterus tissue with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7111-06) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

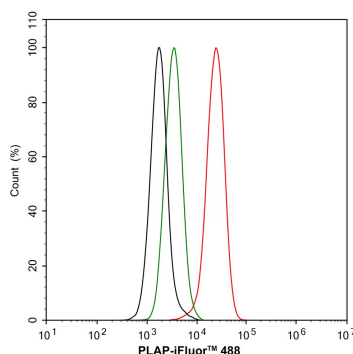
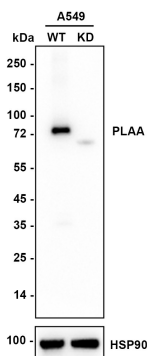


Fig9: Flow cytometric analysis of A549 cells labeling Phospholipase A2 activating protein (PLAP).

Cells were fixed and permeabilized. Then stained with the primary antibody (ET7111-06, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Fig10: Western blot analysis of Phospholipase A2 activating protein (PLAP) on different lysates with Rabbit anti-Phospholipase A2 activating protein (PLAP) antibody (ET7111-06) at 1/1,000 dilution.



Lane 1: A549-parental cell lysate

Lane 2: A549-Phospholipase A2 activating protein (PLAP) KD cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 87 kDa

Observed band size: 87 kDa

Exposure time: 30 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7111-06) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

Background References

1. Gupta P. et. al. Molecular details of secretory phospholipase A2 from flax (*Linum usitatissimum* L.) provide insight into its structure and function. *Sci Rep.* 2017 Sep 11;7(1):11080.
2. Smichi N. et. al. Functional and Structural Characterization of a Thermostable Phospholipase A2 from a Sparidae Fish (*Diplodus annularis*). *J Agric Food Chem.* 2017 Mar 22;65(11):2468-2480.

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