Anti-ALIX Antibody [JM85-31]

ET1705-74

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Cell
Molecular Wt:	Predicted band size: 96 kDa
Clone number:	JM85-31

Description: ALG-2-interacting protein (Alix), also designated programmed cell death 6-interacting protein (PDCD6-interacting protein), is a cytoplasmic protein. Alix interacts with apoptosis-associated proteins (ALG-2 and PDCD6) and with the endocytosis-regulator CIN85. Additionally, Alix interacts with the endosomal sorting complexes required for transport (ESCRT) proteins (Tsg101 and CHMP4) and can associate with HIV-1. The endophilins (SH3P4, SH3P8 and SH3P13), enzymes that change curvature of the membrane that are required for early and late steps of coated vesicle formation, also bind to Alix. Alix is involved in the concentration and sorting of cargo proteins of the multivesicular body for incorporation into vesicles.

Immunogen: Recombinant protein within Human ALIX aa 1-160 / 868.

Positive control: HEK-293 cell lysate, K-562 cell lysate, Jurkat cell lysate, PC-3M cell lysate, MCF7 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, mouse liver tissue lysate, rat liver tissue lysate, rat stomach tissue, human colon carcinoma tissue, human prostate tissue, MCF7, NIH/3T3, PC-12, HEK-293..

Subcellular location: Cell junction, Cytoplasm, Cytoskeleton, Secreted, Tight junction.

Database links: SwissProt: Q8WUM4 Human | Q9WU78 Mouse | Q9QZA2 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:50-1:200
FC	1:1,000
IF-Cell	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

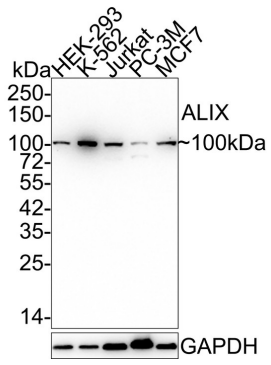

Fig1: Western blot analysis of ALIX on different lysates with Rabbit anti-ALIX antibody (ET1705-74) at 1/2,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: K-562 cell lysate
Lane 3: Jurkat cell lysate
Lane 4: PC-3M cell lysate
Lane 5: MCF7 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 96 kDa
Observed band size: 100 kDa

Exposure time: 5 minutes;

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 (ET1705-74) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

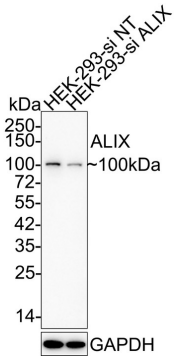

Fig2: Western blot analysis of ALIX on different lysates with Rabbit anti-ALIX antibody (ET1705-74) at 1/2,000 dilution.

Lane 1: HEK-293-si NT cell lysate
Lane 2: HEK-293-si ALIX cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 96 kDa
Observed band size: 100 kDa

Exposure time: 5 minutes;

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 (ET1705-74) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

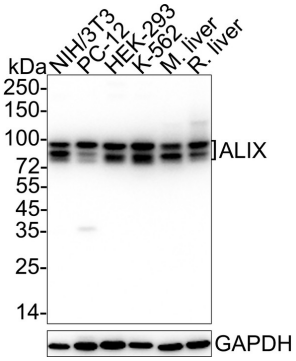

Fig3: Western blot analysis of ALIX on different lysates with Rabbit anti-ALIX antibody (ET1705-74) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: PC-12 cell lysate (20 µg/Lane)
Lane 3: HEK-293 cell lysate (20 µg/Lane)
Lane 4: K-562 cell lysate (20 µg/Lane)
Lane 5: Mouse liver tissue lysate (30 µg/Lane)
Lane 6: Rat liver tissue lysate (30 µg/Lane)

Predicted band size: 96 kDa
Observed band size: 80-100 kDa

Exposure time: 6 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 (ET1705-74) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

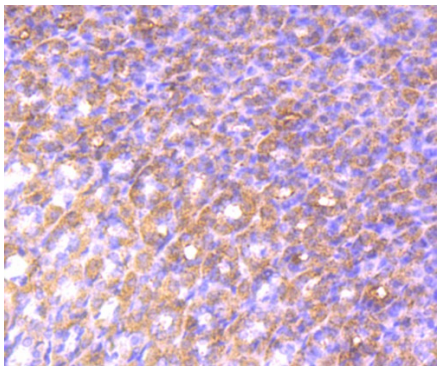

Fig4: Immunohistochemical analysis of paraffin-embedded rat stomach tissue using anti-ALIX antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-74, 1/50) for 30 minutes 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.

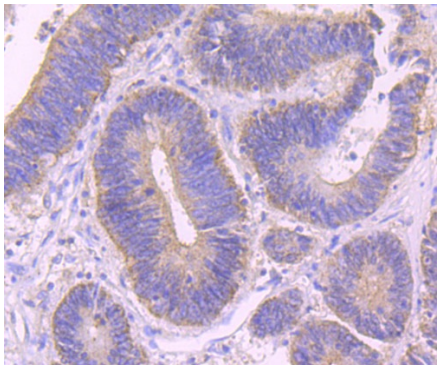

Fig5: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-ALIX antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-74, 1/50) for 30 minutes 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.

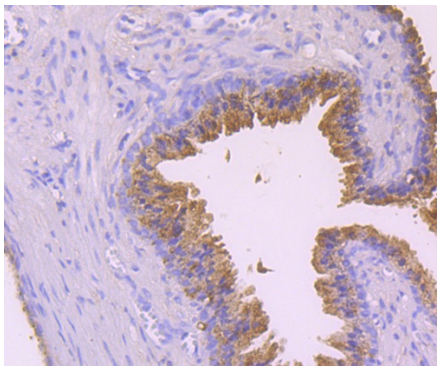

Fig6: Immunohistochemical analysis of paraffin-embedded human prostate tissue using anti-ALIX antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-74, 1/50) for 30 minutes 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.

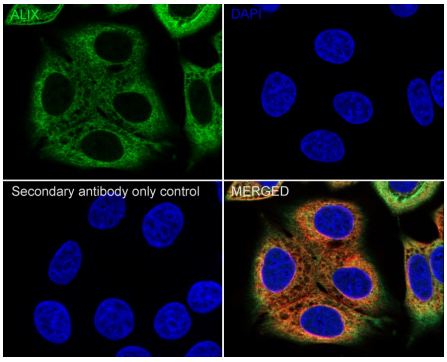

Fig7: Immunocytochemistry analysis of MCF7 cells labeling ALIX with Rabbit anti-ALIX antibody (ET1705-74) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-ALIX antibody (ET1705-74) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

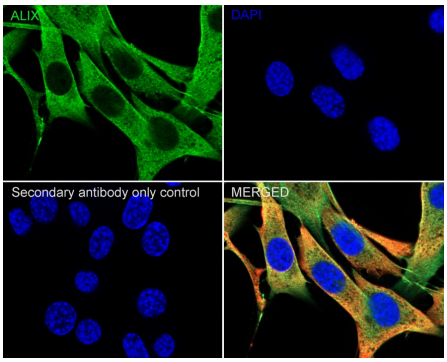

Fig8: Immunocytochemistry analysis of NIH/3T3 cells labeling ALIX with Rabbit anti-ALIX antibody (ET1705-74) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-ALIX antibody (ET1705-74) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

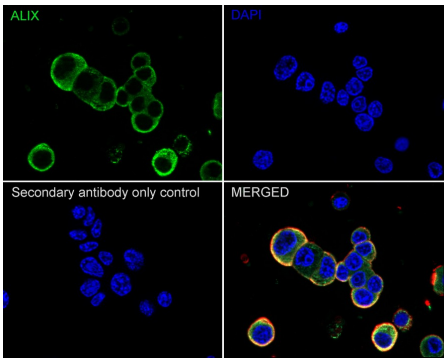

Fig9: Immunocytochemistry analysis of PC-12 cells labeling ALIX with Rabbit anti-ALIX antibody (ET1705-74) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-ALIX antibody (ET1705-74) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

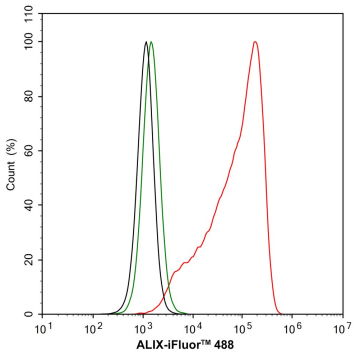

Fig10: Flow cytometric analysis of HEK-293 cells labeling ALIX.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-74, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

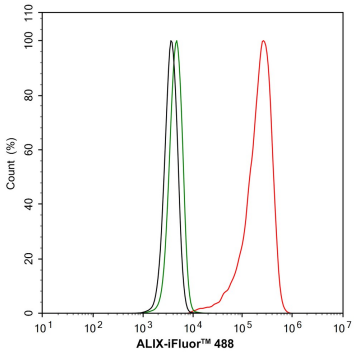

Fig11: Flow cytometric analysis of NIH/3T3 cells labeling ALIX.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-74, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

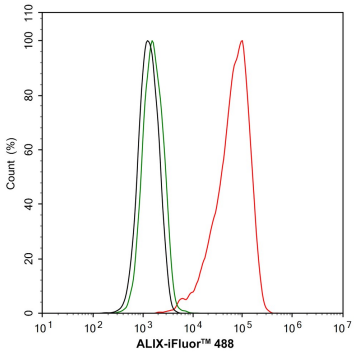

Fig12: Flow cytometric analysis of PC-12 cells labeling ALIX.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-74, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Wiklander OP et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. J Extracell Vesicles 4:26316 (2015).
2. Cypryk, W.et al. Proteomic and Bioinformatic Characterization of Extracellular Vesicles Released from Human Macrophages upon Influenza A Virus Infection. J. Proteome Res. 16: 217-227 (2017).