Anti-LAMP2 Antibody [D3-D8-D3]

EM1901-62



Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 45 kDa
Clone number:	D3-D8-D3
Description:	Plays an important role in chaperone-mediated autophagy, a process that mediates lysosomal degradation of proteins in response to various stresses and as part of the normal turnover of proteins with a long biological half-live. Functions by binding target proteins, such as GAPDH and MLLT11, and targeting them for lysosomal degradation. Plays a role in lysosomal protein degradation in response to starvation (By similarity). Required for the fusion of autophagosomes with lysosomes during autophagy. Cells that lack LAMP2 express normal levels of VAMP8, but fail to accumulate STX17 on autophagosomes, which is the most likely explanation for the lack of fusion between autophagosomes. Required for normal degradation of the contents of autophagosomes. Required for efficient MHCII-mediated presentation of exogenous antigens via its function in lysosomal protein degradation; antigenic peptides generated by proteases in the endosomal/lysosomal compartment are captured by nascent MHCII subunits. Is not required for efficient MHCII-mediated presentation of endogenous antigens.
lmmunogen:	Recombinant protein within human LAMP2 aa 1-300.
Positive control:	HeLa cell lysate, Jurkat cell lysate, HepG2 cell lysate, HUVEC cell lysate, JAR cell lysate, HEK-293 cell lysate, THP-1 cell lysate, HUVEC, human kidney tissue, human liver tissue.
Subcellular location:	Cell membrane, lysosome membrane, endosome membrane, autophagosome membrane.
Database links:	SwissProt: P13473 Human
Recommended Dilutions: WB IHC-P IF-Cell	1:1,000 1:20,000 1:100
Storage Buffer:	1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!\!\mathbb{C}$ after thawing. Aliquot store at -20 $^\circ\!\!\mathbb{C}$. Avoid repeated freeze / thaw cycles.
Purity:	Protein G affinity purified.

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Images



Fig1: Western blot analysis of LAMP2 on different lysates with Mouse anti-LAMP2 antibody (EM1901-62) at 1/1,000 dilution.

Lane 1: HeLa cell lysate Lane 2: Jurkat cell lysate Lane 3: HepG2 cell lysate Lane 4: HUVEC cell lysate Lane 5: JAR cell lysate Lane 6: HEK-293 cell lysate Lane 7: THP-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 45 kDa Observed band size: 110 kDa

Exposure time: 1 minute 2 seconds;

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 (EM1901-62) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of LAMP2 on different lysates with Mouse anti-LAMP2 antibody (EM1901-62) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate Lane 2: HAP1-LAMP2 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 45 kDa Observed band size: 110 kDa

Exposure time: 3 minutes; 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 (EM1901-62) at 1/2,000 dilution was used in primary antibody dilution (K1803) at 4° C overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

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Fig3: Immunocytochemistry analysis of HUVEC cells labeling LAMP2 with Mouse anti-LAMP2 antibody (EM1901-62) at 1/100 dilution.

Cells were fixed in 100% precooled methanol 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 Mouse anti-LAMP2 antibody (EM1901-62) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluorTM 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at $+4^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 1594, HA1122) were used as the secondary antibody at 1/1,000 dilution.



Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-LAMP2 antibody (EM1901-62) at 1/20,000 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 (EM1901-62) at 1/20,000 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 human liver tissue with Mouse anti-LAMP2 antibody (EM1901-62) at 1/20,000 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 (EM1901-62) at 1/20,000 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.

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

Background References

- 1. Dubois A. et. al. LAMP2 expression dictates azacytidine response and prognosis in MDS/AML. Leukemia. 2019 Jun;33(6):1501-1513.
- 2. Zheng H. et. al. Lamp2 inhibits epithelial-mesenchymal transition by suppressing Snail expression in HCC. Oncotarget. 2018 Jul 13;9(54):30240-30252.

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