Anti-SQSTM1 / p62 Antibody [PS00-61]

HA721171

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC, IF-Tissue, IHC-Fr
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	PS00-61

Description: Autophagy receptor required for selective macroautophagy (aggrephagy). Functions as a bridge between polyubiquitinated cargo and autophagosomes. Interacts directly with both the cargo to become degraded and an autophagy modifier of the MAP1 LC3 family. Along with WDFY3, involved in the formation and autophagic degradation of cytoplasmic ubiquitin-containing inclusions (p62 bodies, ALIS/aggresome-like induced structures). Along with WDFY3, required to recruit ubiquitinated proteins to PML bodies in the nucleus. May regulate the activation of NFKB1 by TNF-alpha, nerve growth factor (NGF) and interleukin-1. May play a role in titin/TTN downstream signaling in muscle cells. May regulate signaling cascades through ubiquitination. Adapter that mediates the interaction between TRAF6 and CYLD. May be involved in cell differentiation, apoptosis, immune response and regulation of K+ channels. Involved in endosome organization by retaining vesicles in the perinuclear cloud: following ubiquitination by RNF26, attracts specific vesicle-associated adapters, forming a molecular bridge that restrains cognate vesicles in the perinuclear region and organizes the endosomal pathway for efficient cargo transport. Promotes relocalization of 'Lys-63'-linked ubiquitinated STING1 to autophagosomes. Acts as an activator of the NFE2L2/NRF2 pathway via interaction with KEAP1: interaction inactivates the BCR(KEAP1) complex, promoting nuclear accumulation of NFE2L2/NRF2 and subsequent expression of cytoprotective genes.

Immunogen: Synthetic peptide within Human SQSTM1/ p62 aa 400 to the C-terminus

Positive control: HeLa cell lysate, HeLa treated with 50μM Chloroquine for 18 hours cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, PC-12 cell lysate, SH-SY5Y cell lysate, PANC-1 cell lysate, C2C12, PC-12, A549, human colon carcinoma tissue, human liver tissue, mouse liver tissue, rat liver tissue, mouse brain tissue, rat brain tissue.

Subcellular location: Cytoplasm, Endoplasmic reticulum, Endosome, Lysosome, Nucleus.

Database links: SwissProt: Q13501 Human | Q64337 Mouse | O08623 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IHC-P	1:1,000-1:2,000
IF-Cell	1:100-1:1,000
FC	1:1,000
IF-Tissue	1:200-1:1,000
IHC-Fr	1:500

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

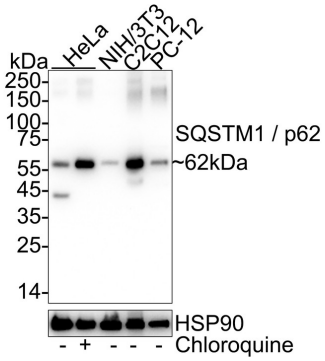

Fig1: Western blot analysis of SQSTM1 / p62 on different lysates with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/10,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 50μM Chloroquine for 18 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: C2C12 cell lysate
Lane 5: PC-12 cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 48 kDa
Observed band size: 62 kDa

Exposure time: 2 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 (HA721171) at 1/10,000 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.

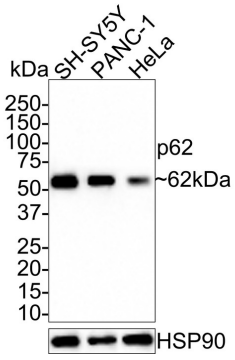

Fig2: Western blot analysis of SQSTM1 / p62 on different lysates with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/5,000 dilution.

Lane 1: SH-SY5Y cell lysate
Lane 2: PANC-1 cell lysate
Lane 3: HeLa cell lysate

Lysates/proteins at 10 μg/Lane.

Predicted band size: 48 kDa
Observed band size: 62 kDa

Exposure time: 1 minute; 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 (HA721171) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

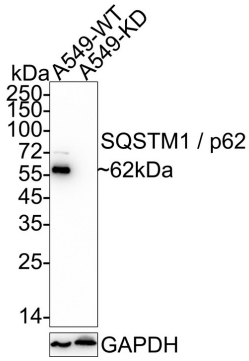

Fig3: Western blot analysis of SQSTM1 / p62 on different lysates with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si SQSTM1 / p62 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 62 kDa

Exposure time: 17 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 (HA721171) 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.

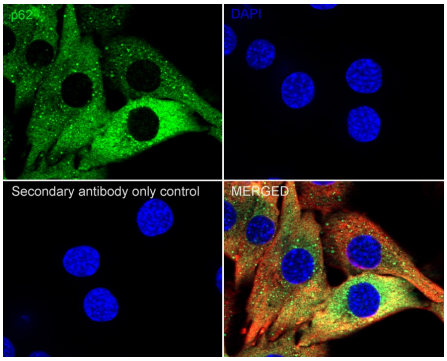

Fig4: Immunocytochemistry analysis of C2C12 cells labeling SQSTM1 / p62 with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

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-SQSTM1 / p62 antibody (HA721171) at 1/1,000 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.

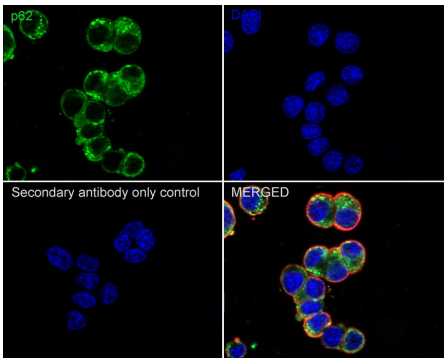

Fig5: Immunocytochemistry analysis of PC-12 cells labeling SQSTM1 / p62 with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/100 dilution.

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-SQSTM1 / p62 antibody (HA721171) 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.

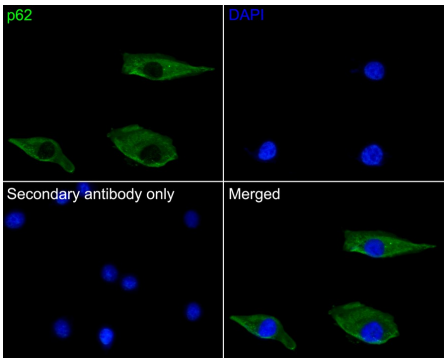

Fig6: Immunocytochemistry analysis of A549 cells labeling SQSTM1 / p62 with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/50 dilution in 2% negative goat serum 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.

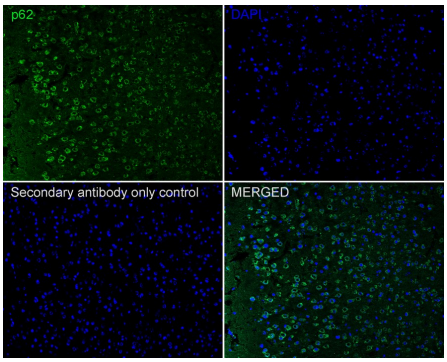

Fig7: Immunofluorescence analysis of frozen mouse brain tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721171, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

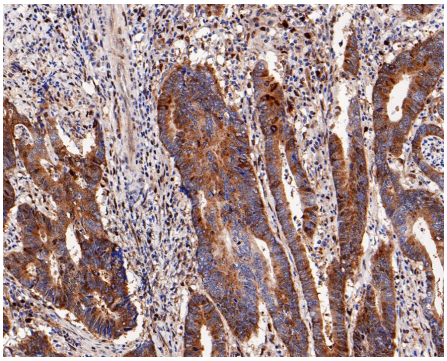

Fig8: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721171) at 1/400 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.

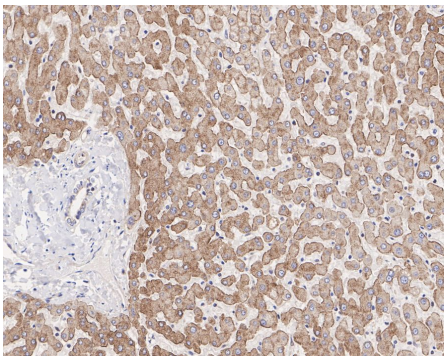

Fig9: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721171) at 1/1,000 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.

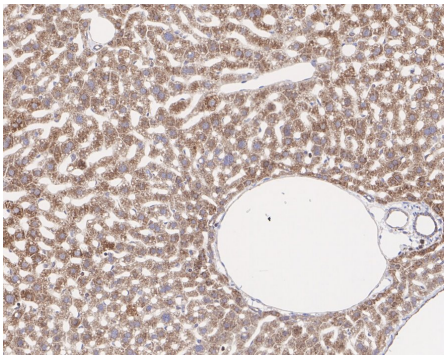

Fig10: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721171) at 1/1,000 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.

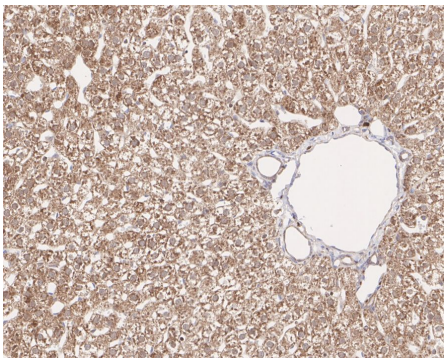

Fig11: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721171) at 1/1,000 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.

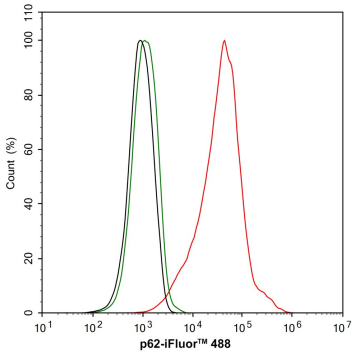

Fig12: Flow cytometric analysis of PC-12 cells labeling SQSTM1 / p62.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721171, 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).

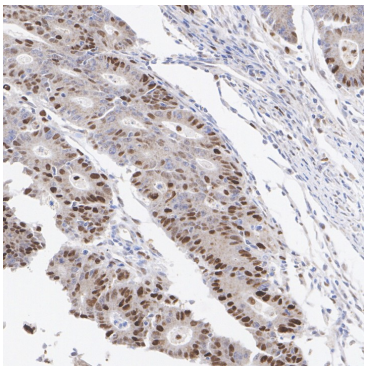

Fig13: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721171) at 1/1,000 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.

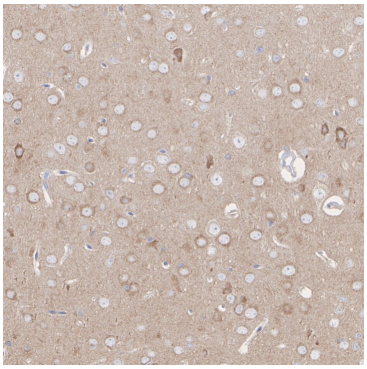

Fig14: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721171) at 1/1,000 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.

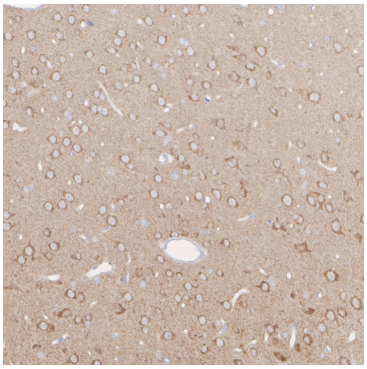

Fig15: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-SQSTM1 / p62 antibody (HA721171) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721171) at 1/1,000 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.

Background References

1. Tan C.T., Chang H.C., Zhou Q., Yu C., Fu N.Y., Sabapathy K., Yu V.C. MOAP-1-mediated dissociation of p62/SQSTM1 bodies releases Keap1 and suppresses Nrf2 signaling. EMBO Rep. 22:e50854-e50854(2021)
2. Prabakaran T., Bodda C., Krapp C., Zhang B.C., Christensen M.H., Sun C., Reinert L., Cai Y., Jensen S.B., Paludan S.R. Attenuation of cGAS-STING signaling is mediated by a p62/SQSTM1-dependent autophagy pathway activated by TBK1. EMBO J. 37:0-0(2018)