Anti-Phospho-SQSTM1 / p62 (S349) Antibody [PSH08-97] - BSA and Azide free

HA751258

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	PSH08-97

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 phospho-peptide corresponding to residues surrounding Ser349 of Human SQSTM1/ p62.

Positive control: HeLa treated with 2μM MG-132 for 18 hours cell lysate, NIH/3T3 treated with 10μM MG-132 for 8 hours cell lysate, HeLa cells treated with 2μM MG-132 for 18 hours, NIH/3T3cells treated with 10μM MG-132 for 8 hours , human cerebrum tissue.

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

Database links: SwissProt: Q13501 Human | Q64337 Mouse

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:5,000
IHC-P	1:30,000

Storage Buffer: 1*PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

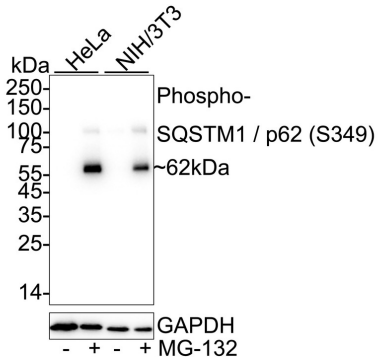

Fig1: Western blot analysis of Phospho-SQSTM1 / p62 (S349) on different lysates with Rabbit anti-Phospho-SQSTM1 / p62 (S349) antibody (HA751258) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 2μM MG-132 for 18 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 10μM MG-132 for 8 hours cell lysate
Lysates/proteins at 20 μg/Lane.

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

Exposure time: 11 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 (HA751258) 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.

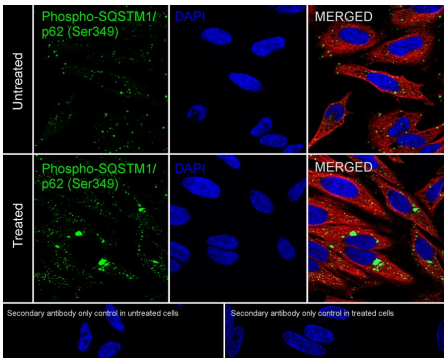

Fig2: Immunocytochemistry analysis of HeLa cells treated with 2μM MG-132 for 18 hours labeling Phospho-SQSTM1 / p62 (S349) with Rabbit anti-Phospho-SQSTM1 / p62 (S349) antibody (HA751258) at 1/5,000 dilution.

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-Phospho-SQSTM1 / p62 (S349) antibody (HA751258) at 1/5,000 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 (HA601187, 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.

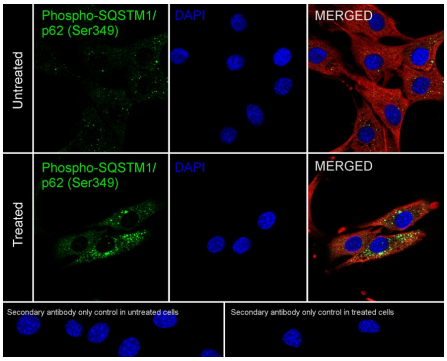

Fig3: Immunocytochemistry analysis of NIH/3T3 cells treated with 10μM MG-132 for 8 hours labeling Phospho-SQSTM1 / p62 (S349) with Rabbit anti-Phospho-SQSTM1 / p62 (S349) antibody (HA751258) at 1/5,000 dilution.

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-Phospho-SQSTM1 / p62 (S349) antibody (HA751258) at 1/5,000 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 (HA601187, 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.

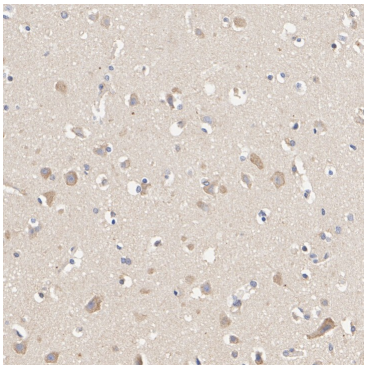

Fig4: Immunohistochemical analysis of paraffin-embedded human cerebrum tissue with Rabbit anti-Phospho-SQSTM1 / p62 (S349) antibody (HA751258) at 1/30,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA751258) at 1/30,000 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)