

SQSTM1 (p62) Antibody (C-term) Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP2183B

Specification

SQSTM1 (p62) Antibody (C-term) - Product Information

Application Primary Accession Other Accession Reactivity Predicted Host Clonality Isotype Antigen Region WB, IF, IHC-P,E <u>Q13501</u> <u>Q08623</u>, <u>Q64337</u> Human, Mouse Rat Rabbit Polyclonal Rabbit IgG 317-346

SQSTM1 (p62) Antibody (C-term) - Additional Information

Gene ID 8878

Other Names

Sequestosome-1, EBI3-associated protein of 60 kDa, EBIAP, p60, Phosphotyrosine-independent ligand for the Lck SH2 domain of 62 kDa, Ubiquitin-binding protein p62, SQSTM1, ORCA, OSIL

Target/Specificity

This SQSTM1 (p62) antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 317-346 amino acids of human SQSTM1 (p62).

Dilution WB~~1:2000 IF~~1:50~100 IHC-P~~1:25 E~~Use at an assay dependent concentration.

Format

Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein A column, followed by peptide affinity purification.

Storage

Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

Precautions

SQSTM1 (p62) Antibody (C-term) is for research use only and not for use in diagnostic or therapeutic procedures.

SQSTM1 (p62) Antibody (C-term) - Protein Information



Name SQSTM1 {ECO:0000303|PubMed:16286508, ECO:0000312|HGNC:HGNC:11280}

Function Molecular adapter required for selective macroautophagy (aggrephagy) by acting as a bridge between polyubiguitinated proteins and autophagosomes (PubMed: 15340068, PubMed: 15953362, PubMed: 16286508, PubMed: 17580304, PubMed: 20168092, PubMed:22017874, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:29343546, PubMed:29507397, PubMed:31857589, PubMed:33509017, PubMed:34471133, PubMed:34893540, PubMed:35831301, PubMed:37306101, PubMed: 37802024). Promotes the recruitment of ubiquitinated cargo proteins to autophagosomes via multiple domains that bridge proteins and organelles in different steps (PubMed: 16286508, PubMed:20168092, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:29343546, PubMed:29507397, PubMed:34893540, PubMed:37802024). SQSTM1 first mediates the assembly and removal of ubiquitinated proteins by undergoing liquid-liquid phase separation upon binding to ubiquitinated proteins via its UBA domain, leading to the formation of insoluble cytoplasmic inclusions, known as p62 bodies (PubMed: 15911346, PubMed: 20168092, PubMed:22017874, PubMed:24128730, PubMed:29343546, PubMed:29507397, PubMed:<u>31857589</u>, PubMed:<u>37802024</u>). SQSTM1 then interacts with ATG8 family proteins on autophagosomes via its LIR motif, leading to p62 body recruitment to autophagosomes, followed by autophagic clearance of ubiquitinated proteins (PubMed: 16286508, PubMed: 17580304, PubMed:20168092, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed: 37802024). SQSTM1 is itself degraded along with its ubiguitinated cargos (PubMed: 16286508, PubMed: 17580304, PubMed: 37802024). Also required to recruit ubiquitinated proteins to PML bodies in the nucleus (PubMed: 20168092). Also involved in autophagy of peroxisomes (pexophagy) in response to reactive oxygen species (ROS) by acting as a bridge between ubiguitinated PEX5 receptor and autophagosomes (PubMed: 26344566). Acts as an activator of the NFE2L2/NRF2 pathway via interaction with KEAP1: interaction inactivates the BCR(KEAP1) complex by sequestering the complex in inclusion bodies, promoting nuclear accumulation of NFE2L2/NRF2 and subsequent expression of cytoprotective genes (PubMed:20452972, PubMed:28380357, PubMed:33393215, PubMed:37306101). Promotes relocalization of 'Lys-63'-linked ubiquitinated STING1 to autophagosomes (PubMed: 29496741). Involved in endosome organization by retaining vesicles in the perinuclear cloud: following ubiguitination 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 (PubMed:27368102, PubMed:33472082). Sequesters tensin TNS2 into cytoplasmic puncta, promoting TNS2 ubiguitination and proteasomal degradation (PubMed: 25101860). May regulate the activation of NFKB1 by TNF-alpha, nerve growth factor (NGF) and interleukin-1 (PubMed: 10356400, PubMed: 10747026, PubMed: 11244088, PubMed:12471037, PubMed:16079148, PubMed:19931284). May play a role in titin/TTN downstream signaling in muscle cells (PubMed: 15802564). Adapter that mediates the interaction between TRAF6 and CYLD (By similarity).

Cellular Location

Cytoplasmic vesicle, autophagosome. Preautophagosomal structure. Cytoplasm, cytosol. Nucleus, PML body. Late endosome. Lysosome. Nucleus Endoplasmic reticulum. Cytoplasm, myofibril, sarcomere {ECO:0000250|UniProtKB:008623}. Note=In cardiac muscle, localizes to the sarcomeric band (By similarity). Localizes to cytoplasmic membraneless inclusion bodies, known as p62 bodies, containing polyubiquitinated protein aggregates (PubMed:11786419, PubMed:20357094, PubMed:22017874, PubMed:29343546, PubMed:29507397, PubMed:31857589, PubMed:37306101, PubMed:37802024). In neurodegenerative diseases, detected in Lewy bodies in Parkinson disease, neurofibrillary tangles in Alzheimer disease, and HTT aggregates in Huntington disease (PubMed:15158159). In protein aggregate diseases of the liver, found in large amounts in Mallory bodies of alcoholic and nonalcoholic steatohepatitis, hyaline bodies in hepatocellular carcinoma, and in SERPINA1 aggregates (PubMed:11981755) Enriched in Rosenthal fibers of pilocytic astrocytoma (PubMed:11786419). In the cytoplasm, observed in both membrane-free ubiquitin-containing protein aggregates (sequestosomes) and membrane- surrounded autophagosomes (PubMed:15953362, PubMed:17580304) Colocalizes with TRIM13 in the perinuclear endoplasmic reticulum (PubMed:22178386). Co-localizes with TRIM5 in



cytoplasmic bodies (PubMed:20357094). When nuclear export is blocked by treatment with leptomycin B, accumulates in PML bodies (PubMed:20168092) {ECO:0000250|UniProtKB:O08623, ECO:0000269|PubMed:11786419, ECO:0000269|PubMed:11981755, ECO:0000269|PubMed:15158159, ECO:0000269|PubMed:15953362, ECO:0000269|PubMed:17580304, ECO:0000269|PubMed:20168092, ECO:0000269|PubMed:20357094, ECO:0000269|PubMed:22017874, ECO:0000269|PubMed:22178386, ECO:0000269|PubMed:29343546, ECO:0000269|PubMed:29507397, ECO:0000269|PubMed:31857589, ECO:0000269|PubMed:37306101, ECO:0000269|PubMed:37802024}

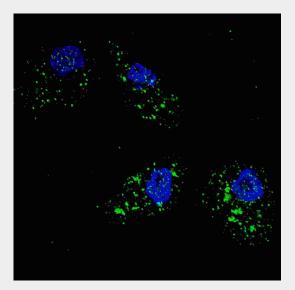
Tissue Location Ubiguitously expressed.

SQSTM1 (p62) Antibody (C-term) - Protocols

Provided below are standard protocols that you may find useful for product applications.

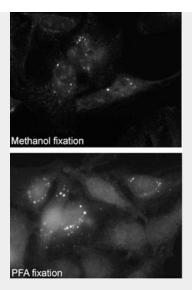
- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

SQSTM1 (p62) Antibody (C-term) - Images

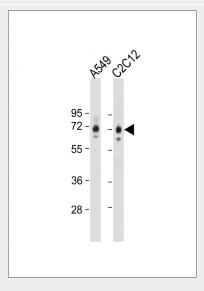


Fluorescent image of U251 cells stained with SQSTM1 (p62) (C-term) antibody. U251 cells were treated with Chloroquine (50 μ M,16h), then fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP2183b SQSTM1 (p62) (C-term) primary antibody (1:200, 2 h at room temperature). For secondary antibody, Alexa Fluor® 488 conjugated donkey anti-rabbit antibody (green) was used (1:1000, 1h). Nuclei were counterstained with Hoechst 33342 (blue) (10 μ g/ml, 5 min). SQSTM1 (p62) immunoreactivity is localized to autophagic vacuoles in the cytoplasm of U251 cells, supported by Human Protein Atlas Data (http://www.proteinatlas.org/ENSG00000161011).



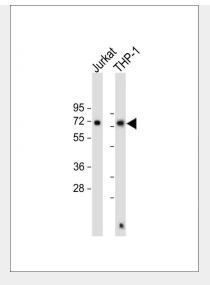


Immunofluorescence staining of Autophagy SQSTM1 (p62) Antibody (C-term) (Cat# AP2183b) on Methanol-fixed and PFA fixed HeLa cells. Data courtesy of Dr. Eeva-Liisa Eskelinen, University of Helsinki, Finland.

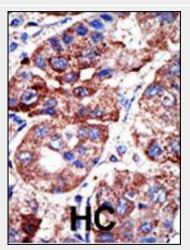


All lanes : Anti-SQSTM1 (p62) Antibody (C-term) at 1:2000 dilution Lane 1: A549 whole cell lysate Lane 2: C2C12 whole cell lysate Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 48 kDa Blocking/Dilution buffer: 5% NFDM/TBST.



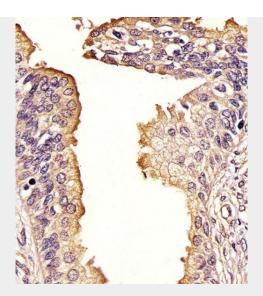


All lanes : Anti-SQSTM1 (p62) Antibody (C-term) at 1:2000 dilution Lane 1: Jurkat whole cell lysate Lane 2: THP-1 whole cell lysate Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 48 kDa Blocking/Dilution buffer: 5% NFDM/TBST.



Formalin-fixed and paraffin-embedded human cancer tissue reacted with the primary antibody, which was peroxidase-conjugated to the secondary antibody, followed by DAB staining. This data demonstrates the use of this antibody for immunohistochemistry; clinical relevance has not been evaluated. BC = breast carcinoma; HC = hepatocarcinoma.





AP2183b staining SQSTM1 in Human prostate tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.

SQSTM1 (p62) Antibody (C-term) - Background

SQSTM1/p62 is an adapter protein which binds ubiquitin and may regulate the activation of NFKB1 by TNF-alpha, nerve growth factor (NGF) and interleukin-1. This protein may play a role in titin/TTN downstream signaling in muscle cells, and may also regulate signaling cascades through ubiquitination. This protein is involved in cell differentiation, apoptosis, immune response and regulation of K(+) channels. SQSTM1/p62 also appears to play a role in macroautophagic removal of intracellular protein aggregates. Cellular depletion studies of SQSTM1/p62 have indicated a role for association with LC3 and aggregate proteins in order to facilitate normal formation of the autophagosome.

SQSTM1 (p62) Antibody (C-term) - References

References for protein:

1.Seibenhener, M.L., et al., Mol. Cell. Biol. 24(18):8055-8068 (2004).

2.Eekhoff, E.W., et al., Arthritis Rheum. 50(5):1650-1654 (2004).

3.Brajenovic, M., et al., J. Biol. Chem. 279(13):12804-12811 (2004).

4.Kuusisto, E., et al., J. Neuropathol. Exp. Neurol. 62(12):1241-1253 (2003).

5. Johnson-Pais, T.L., et al., J. Bone Miner. Res. 18(10):1748-1753 (2003).

References for U251 cell line:

1. Westermark B.; Pontén J.; Hugosson R. (1973)." Determinants for the establishment of permanent tissue culture lines from human gliomas". Acta Pathol Microbiol Scand A. 81:791-805. [PMID: 4359449].

2. Pontén, J., Westermark B. (1978)." Properties of Human Malignant Glioma Cells in Vitro". Medical Biology 56: 184-193.[PMID: 359950].

3. Geng Y.;Kohli L.; Klocke B.J.; Roth K.A.(2010). "Chloroquine-induced autophagic vacuole accumulation and cell death in glioma cells is p53 independent". Neuro Oncol. 12(5): 473–481.[PMID: 20406898].

SQSTM1 (p62) Antibody (C-term) - Citations

- Zinc oxide nanoparticles induces cell death and consequently leading to incomplete neural tube closure through oxidative stress during embryogenesis
- <u>Cholesteryl hemiazelate causes lysosome dysfunction impacting vascular smooth muscle</u>



cell homeostasis

- Expression and prognostic significance of the DNA damage response pathway and autophagy markers in gastric cancer
- TXNIP/VDUP1 attenuates steatohepatitis via autophagy and fatty acid oxidation
- Rapamycin induces megakaryocytic differentiation through increasing autophagy in Dami cells
- Enteritidis Effector AvrA Suppresses Autophagy by Reducing Beclin-1 Protein
- Axonal autophagosome maturation defect through failure of ATG9A sorting underpins pathology in AP-4 deficiency syndrome
- The p53 inactivators pifithrin-μ and pifithrin-α mitigate TBI-induced neuronal damage through regulation of oxidative stress, neuroinflammation, autophagy and mitophagy.
- <u>Helicobacter pylori cholesterol glucosylation modulates autophagy for increasing</u> <u>intracellular survival in macrophages.</u>
- Genistein and Myd88 Activate Autophagy in High Glucose-Induced Renal Podocytes In Vitro.
- Honokiol inhibits in vitro and in vivo growth of oral squamous cell carcinoma through induction of apoptosis, cell cycle arrest and autophagy.
- <u>Up-regulation of autophagy is a mechanism of resistance to chemotherapy and can be inhibited by pantoprazole to increase drug sensitivity.</u>
- Deletion of the BH3-only protein Noxa alters electrographic seizures but does not protect against hippocampal damage after status epilepticus in mice.
- Role of Autophagy as a Survival Mechanism for Hypoxic Cells in Tumors.
- Interference with HMGB1 increases the sensitivity to chemotherapy drugs by inhibiting HMGB1-mediated cell autophagy and inducing cell apoptosis.
- Effect of pantoprazole to enhance activity of docetaxel against human tumour xenografts by inhibiting autophagy.
- Intestinal epithelial vitamin D receptor deletion leads to defective autophagy in colitis.
- Inhibition of Intracellular Clusterin Attenuates Cell Death in Nephropathic Cystinosis.
- <u>A novel sulindac derivative inhibits lung adenocarcinoma cell growth through suppression of Akt/mTOR signaling and induction of autophagy.</u>
- Potent obatoclax cytotoxicity and activation of triple death mode killing across infant acute lymphoblastic leukemia.
- p62/SQSTM1 prominently accumulates in renal proximal tubules in nephropathic cystinosis.
- <u>Curcumin induces autophagy to protect vascular endothelial cell survival from oxidative</u> stress damage.
- <u>Increased hippocampal accumulation of autophagosomes predicts short-term recognition</u> <u>memory impairment in aged mice.</u>
- Induction of an incomplete autophagic response by cancer-preventive geranylgeranoic acid (GGA) in a human hepatoma-derived cell line.
- Overexpression of the autophagic beclin-1 protein clears mutant ataxin-3 and alleviates Machado-Joseph disease.
- Autophagy negatively regulates keratinocyte inflammatory responses via scaffolding protein p62/SQSTM1.
- Roles of SIRT1 in the acute and restorative phases following induction of inflammation.
- Invasion and multiplication of Helicobacter pylori in gastric epithelial cells and implications for antibiotic resistance.
- Autophagy induction with RAD001 enhances chemosensitivity and radiosensitivity through <u>Met inhibition in papillary thyroid cancer.</u>
- Epidermal growth factor reduces autophagy in intestinal epithelium and in the rat model of necrotizing enterocolitis.
- Helicobacter pylori impairs murine dendritic cell responses to infection.
- Transcription factor GATA4 inhibits doxorubicin-induced autophagy and cardiomyocyte death.
- Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling.
- Impaired protein aggregate handling and clearance underlie the pathogenesis of



p97/VCP-associated disease.