

# SQSTM1 (p62) Antibody (C-term)

Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP2183B

## **Product Information**

Application Primary Accession Other Accession Reactivity Predicted Host	WB, IHC-P, IF, E <u>Q13501</u> <u>O08623</u> , <u>Q64337</u> Human, Mouse, Rat Mouse, Rat Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Calculated MW	47687
Antigen Region	317-346

#### **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~~1:500 IHC-P~~1:100~500 IF~~1:200 E~~Use at an assay dependent concentration.
Format	Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is prepared by Saturated Ammonium Sulfate (SAS) precipitation followed by dialysis against PBS.
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.

### **Protein Information**

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

Molecular adapter required for selective macroautophagy (aggrephagy) by acting as a bridge between polyubiquitinated 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:31857589, PubMed:37802024). 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:<u>28404643</u>, PubMed:<u>37802024</u>). SQSTM1 is itself degraded along with its ubiquitinated 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 ubiquitinated 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:<u>37306101</u>). Promotes relocalization of 'Lys-63'-linked ubiquitinated STING1 to autophagosomes (PubMed:29496741). 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 (PubMed:27368102, PubMed:33472082). Sequesters tensin TNS2 into cytoplasmic puncta, promoting TNS2 ubiquitination and proteasomal degradation (PubMed:<u>25101860</u>). 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 LocationCytoplasmic vesicle, autophagosome. Preautophagosomal structure.<br/>Cytoplasm, cytosol. Nucleus, PML body. Late endosome. Lysosome. Nucleus<br/>Endoplasmic reticulum. Cytoplasm, myofibril, sarcomere<br/>{ECO:0000250|UniProtKB:008623}. Note=In cardiac muscle, localizes to the<br/>sarcomeric band (By similarity). Localizes to cytoplasmic membraneless<br/>inclusion bodies, known as p62 bodies, containing polyubiquitinated protein<br/>aggregates (PubMed:11786419, PubMed:20357094, PubMed:22017874,<br/>PubMed:29343546, PubMed:29507397, PubMed:31857589,<br/>PubMed:37306101, PubMed:37802024). In neurodegenerative diseases,<br/>detected in Lewy bodies in Parkinson disease, neurofibrillary tangles in<br/>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 membranesurrounded 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:008623, 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** 

Ubiquitously expressed.

## 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.

#### 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].

#### Images

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.





Fluorescent image of U251 cells stained with SQSTM1 (p62) (C-term) antibody. U251 cells were treated with Chloroquine (50  $\mu$ 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  $\mu$ 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).





95 - -72 - -55 - -36 - -28 - -

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.

Western blot analysis of SQSTM1 Antibody (C331) Pab (Cat.#AP2183b) pre-incubated without(lane 1) and with(lane 2) blocking peptide in MCF-7 cell line lysate. SQSTM1 Antibody (C331) (arrow) was detected using the purified Pab.





Western blot analysis of SQSTM1 (arrow) using rabbit polyclonal Autophagy SQSTM1 (p62) Antibody (C-term ) (Cat.#AP2183b). 293 cell lysates (2 ug/lane) either nontransfected (Lane 1) or transiently transfected with the SQSTM1 gene (Lane 2) (Origene Technologies).

## Citations

- Cholesteryl hemiazelate causes lysosome dysfunction impacting vascular smooth muscle 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
- Helicobacter pylori cholesterol glucosylation modulates autophagy for increasing intracellular survival in macrophages.
- 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.
- Up-regulation of autophagy is a mechanism of resistance to chemotherapy and can be inhibited by pantoprazole to increase drug sensitivity.
- 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 stress damage.</u>
- Increased hippocampal accumulation of autophagosomes predicts short-term recognition memory impairment in aged mice.
- 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.
- Epidermal growth factor reduces autophagy in intestinal epithelium and in the rat model of necrotizing enterocolitis.
- <u>Autophagy induction with RAD001 enhances chemosensitivity and radiosensitivity through Met inhibition in papillary</u> <u>thyroid cancer.</u>
- 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.

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