

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

Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP2183B

# **Specification**

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

Application IF, WB, IHC-P,E

Primary Accession <u>Q13501</u>

Other Accession
Reactivity
O08623, 064337
Human, Mouse

Predicted Rat
Host Rabbit
Clonality Polyclonal
Isotype Rabbit IgG
Antigen Region 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**

IF~~1:50~100 WB~~1:2000 IHC-P~~1:50~100

#### **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

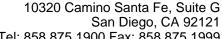
Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

#### **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 a bridge between polyubiquitinated proteins and autophagosomes (PubMed: 16286508, PubMed:20168092, PubMed:24128730, PubMed:34471133, PubMed:22622177, PubMed: 22017874, PubMed: 15340068, PubMed: 17580304, PubMed: 28404643, PubMed: <u>15953362</u>, PubMed: <u>29507397</u>, PubMed: <u>29343546</u>, PubMed: <u>31857589</u>, PubMed:33509017, 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:24128730, PubMed: 22622177, PubMed: 29507397, PubMed: 29343546, PubMed: 28404643, 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:24128730, PubMed:22017874, PubMed: 29507397, PubMed: 29343546, 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:20168092, PubMed:24128730, PubMed:22622177, PubMed: 28404643, PubMed: 17580304, PubMed: 37802024). 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: <u>20452972</u>, PubMed: <u>28380357</u>, PubMed: <u>33393215</u>, 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: 25101860). May regulate the activation of NFKB1 by TNF-alpha, nerve growth factor (NGF) and interleukin-1 (PubMed: 16079148, PubMed: 10747026, PubMed: 10356400, PubMed:11244088, PubMed:19931284, PubMed:12471037). May play a role in titin/TTN downstream signaling in muscle cells (PubMed: 15802564). Adapter that mediates the interaction

# **Cellular Location**

between TRAF6 and CYLD (By similarity).

Cytoplasmic vesicle, autophagosome. Preautophagosomal structure. Cytoplasm, cytosol. Nucleus, PML body. Late endosome. Lysosome. Nucleus Endoplasmic reticulum. Cytoplasm, myofibril, sarcomere {ECO:0000250|UniProtKB:O08623}. 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:29507397, PubMed:29343546, PubMed:37802024, PubMed:31857589, PubMed:37306101). 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:2017874, ECO:0000269|PubMed:22178386, ECO:0000269|PubMed:29343546, ECO:0000269|PubMed:29507397, ECO:0000269|PubMed:31857589, ECO:0000269|PubMed:37306101, ECO:0000269|PubMed:37802024}

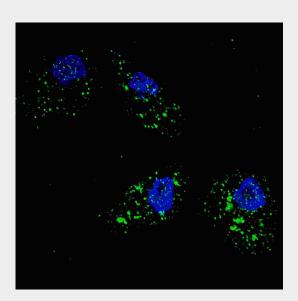
**Tissue Location**Ubiquitously expressed.

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

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

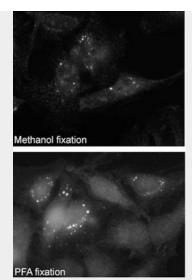
- Western Blot
- Blocking Peptides
- Dot Blot
- <u>Immunohistochemistry</u>
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

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

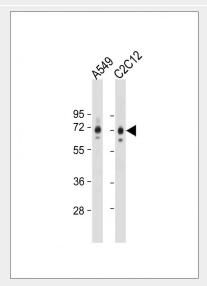


Fluorescent image of U251 cells stained with SQSTM1 (p62) (C-term) antibody. U251 cells were treated with Chloroquine (50  $\mu$ M,16h), then fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP2183b SQSTM1 (p62) (C-term) primary antibody (1:200, 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).



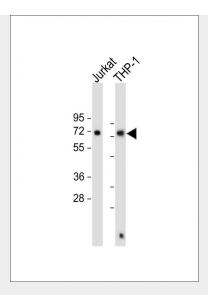


Immunofluorescence staining of Autophagy SQSTM1 (p62) Antibody (C-term) (Cat# AP2183b) on Methanol-fixed and PFA fixed HeLa cells. Data courtesy of Dr. Eeva-Liisa Eskelinen, University of Helsinki, Finland.

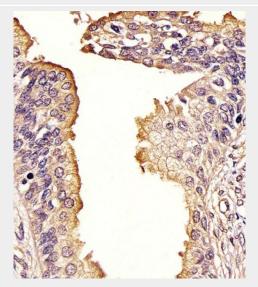


All lanes : Anti-SQSTM1 (p62) Antibody (C-term) at 1:2000 dilution Lane 1: A549 whole cell lysate Lane 2: C2C12 whole cell lysate Lysates/proteins at 20  $\mu$ g per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 48 kDa Blocking/Dilution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



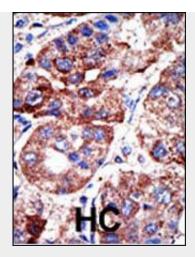


All lanes : Anti-SQSTM1 (p62) Antibody (C-term) at 1:2000 dilution Lane 1: Jurkat whole cell lysate Lane 2: THP-1 whole cell lysate Lysates/proteins at 20  $\mu$ g per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 48 kDa Blocking/Dilution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



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.





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.

# 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].

#### SOSTM1 (p62) Antibody (C-term) - Citations

- 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.
- <u>Up-regulation of autophagy is a mechanism of resistance to chemotherapy and can be inhibited by pantoprazole to increase drug sensitivity.</u>
- <u>Deletion of the BH3-only protein Noxa alters electrographic seizures but does not protect</u> 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.
- A novel sulindac derivative inhibits lung adenocarcinoma cell growth through suppression of Akt/mTOR signaling and induction of autophagy.
- 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.
- Curcumin induces autophagy to protect vascular endothelial cell survival from oxidative stress damage.
- <u>Increased hippocampal accumulation of autophagosomes predicts short-term recognition</u> memory impairment in aged mice.
- <u>Induction of an incomplete autophagic response by cancer-preventive geranylgeranoic acid</u> (GGA) in a human hepatoma-derived cell line.
- Overexpression of the autophagic beclin-1 protein clears mutant ataxin-3 and alleviates Machado-loseph 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.
- Autophagy induction with RAD001 enhances chemosensitivity and radiosensitivity through Met inhibition in papillary thyroid cancer.
- 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.