abcam

Product datasheet

Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] ab280086

KO VALIDATED Recombinant

2 References 6 Images

Overview

Product name	Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND]
Description	Mouse monoclonal [3/P62 LCK LIGAND] to SQSTM1 / p62
Host species	Mouse
Tested applications	**Suitable for:** IP, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1; HeLa; MCF7; HEK-293 whole cell lysates. IHC-P: Human stomach carcinoma, lung carcinoma tissue. ICC/IF: HeLa cells. IP: HeLa whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	3/P62 LCK LIGAND
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab280086 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		1/30.
WB		1/1000. Predicted molecular weight: 47 kDa.
IHC-P		1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/50.

Target

Function Adapter protein which binds ubiquitin and 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 (By similarity). May be involved in cell differentiation, apoptosis, immune response and regulation of K(+) channels.

Tissue specificity Ubiquitously expressed.

Involvement in disease Defects in SQSTM1 are a cause of Paget disease of bone (PDB) [MIM:602080]. PDB is a metabolic bone disease affecting the axial skeleton and characterized by focal areas of increased and disorganized bone turn-over due to activated osteoclasts. Manifestations of the disease include bone pain, deformity, pathological fractures, deafness, neurological complications and increased risk of osteosarcoma. PDB is a chronic disease affecting 2 to 3% of the population above the age of 40 years.

Sequence similarities Contains 1 OPR domain.
Contains 1 UBA domain.
Contains 1 ZZ-type zinc finger.

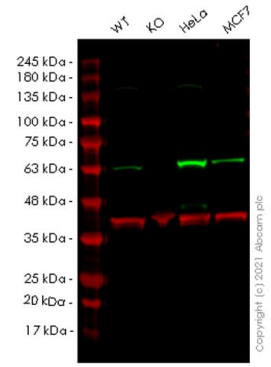
Domain The UBA domain binds specifically 'Lys-63'-linked polyubiquitin chains of polyubiquitinated substrates. Mediates the interaction with TRIM55.
The OPR domain mediates homooligomerization and interactions with PRKCZ, PRKCI, MAP2K5 and NBR1.
The ZZ-type zinc finger mediates the interaction with RIPK1.

Post-translational modifications Phosphorylated. May be phosphorylated by PRKCZ (By similarity). Phosphorylated in vitro by TTN.

Cellular localization Cytoplasm. Late endosome. Nucleus. Sarcomere (By similarity). In cardiac muscles localizes to the sarcomeric band (By similarity). Localizes to late endosomes. May also localize to the nucleus. Accumulates in neurofibrillary tangles and in Lewy bodies of neurons from individuals with Alzheimer and Parkinson disease respectively. Enriched in Rosenthal fibers of pilocytic astrocytoma. In liver cells, accumulates in Mallory bodies associated with alcoholic hepatitis, Wilson disease, indian childhood cirrhosis and in hyaline bodies associated with hepatocellular

carcinoma.

Images



Western blot - Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086)

All lanes : Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086) at 1/1000 dilution

Lane 1 : Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell line), whole cell lysate
Lane 2 : SQSTM1 knockout HAP1 (human chronic myelogenous leukemia near-haploid cell line), whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 4 : MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (IRDye® 800CW) (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) (**ab216777**) at 1/10000 dilution

Predicted band size: 47 kDa
Observed band size: 62 kDa

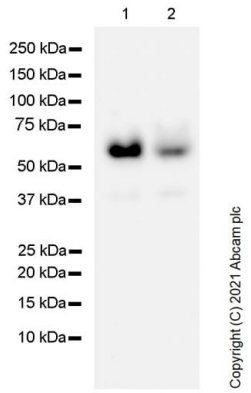
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lanes 1 - 4: Merged signal (red and green). Green - ab280086 observed at 62 kDa. Red - loading control **ab181602** (Rabbit monoclonal [EPR16891] to GAPDH) observed at 36 kDa.

Lanes 1-2: ab280086 Anti-SQSTM1/p62 antibody was shown to react with SQSTM1 in HAP1 cells in Western blot. Loss of signal was observed when SQSTM1 knockout sample was used. Wild-type and SQSTM1 knockout samples were subjected to SDS-PAGE. ab280086 and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated at 4°C overnight at 1/1000 dilution and 1/20000 dilution respectively.

Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680CW) preadsorbed (**ab216777**) and Goat anti-Mouse IgG H&L (IRDye® 800RD) preadsorbed (**ab216772**) secondary antibodies

at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086)

All lanes : Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 2 : HEK-293 (human embryonic kidney epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

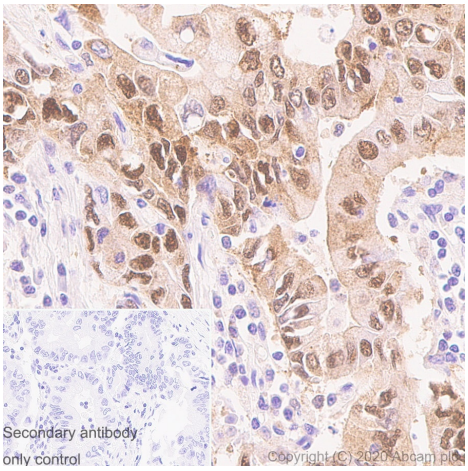
Predicted band size: 47 kDa
Observed band size: 62 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 24086455).

Lysates were made freshly and used in WB immediately to minimize protein degradation.

Exposure time: 15 seconds

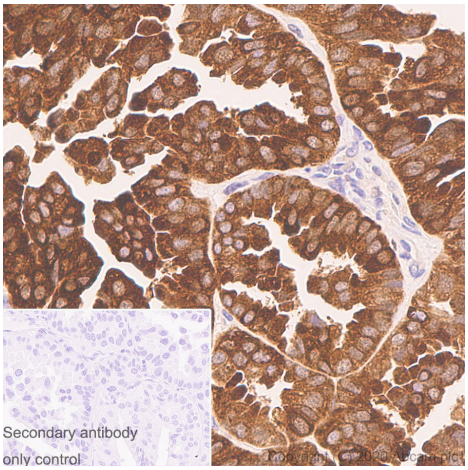


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086)

Immunohistochemical analysis of paraffin-embedded Human stomach carcinoma tissue labeling SQSTM1 / p62 with ab280086 at 1/1000 dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human stomach carcinoma. The section was incubated with ab280086 for 30 mins at room temperature and followed by mouse specific IgG antibody (**ab125913**) for 8mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086)

Immunohistochemical analysis of paraffin-embedded Human lung carcinoma tissue labeling SQSTM1 / p62 with ab280086 at 1/1000 dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human lung carcinoma. The section was incubated with ab280086 for 30 mins at room temperature and followed by mouse specific IgG antibody (**ab125913**) for 8mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

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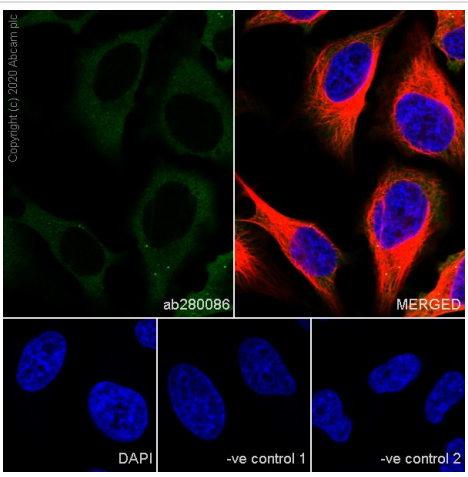
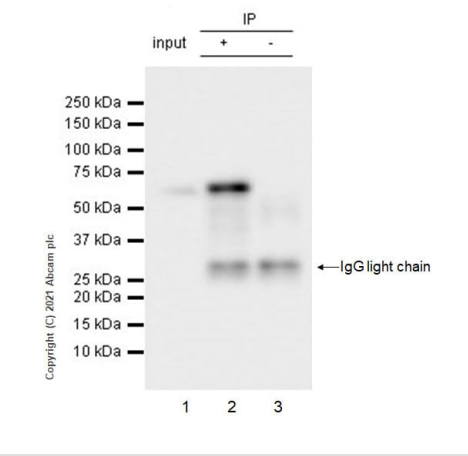


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling SQSTM1 / p62 with ab280086 at 1/50 dilution, followed by **ab150113** Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HeLa cell line. **ab179513** Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution followed by **ab150080** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594)(Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150113** Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [3/P62 LCK LIGAND] (ab280086)

SQSTM1 / p62 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate 10 ug with ab280086 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab280086 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate 10 ug

Lane 2: ab280086 IP in HeLa whole cell lysate

Lane 3: Mouse monoclonal IgG1(**ab18443**) instead of ab280086 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 26 seconds

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