abcam

ab219581

Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR4844
Purification technique	Affinity purification Protein A
Specificity	This antibody got too weak band in rat tissues, you may need to optimize experimental protocols (increasing lysate amount, using lower dilution or higher sensitivity ECL substrate) to get visible band. However, it performs very well in rat cell lines.
Concentration	1.004 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Rat
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Rat, Mouse, Human
Dilution info	-
Notes	-

IP

Tested

Species	Rat, Mouse, Human
Dilution info	-
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-

Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information SQSTM1

Function

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:28404643, 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: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 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, 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).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab219581 is the carrier-free version of ab109012.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

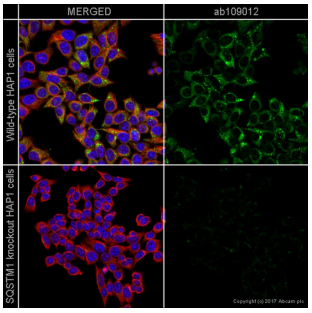
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

22 product images

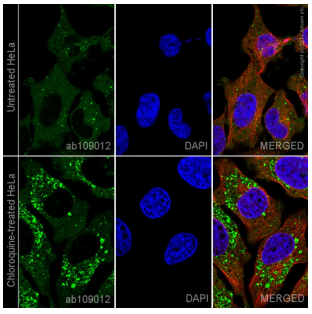


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SQSTM1 / p62 antibody

Purified ab109012 staining SQSTM1 in wild-type HAP1 cells (top panel) and SQSTM1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109012 at 1µg/ml and ab195889 at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109012).

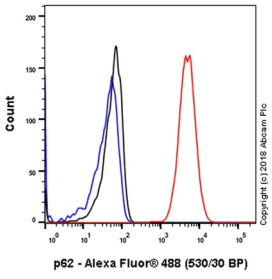


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SQSTM1 / p62 antibody

Purified ab109012 staining SQSTM1/p62 in HeLa cells +/- Chloroquine (50µM, 24 hours). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab109012 at 1µg/ml and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red) followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

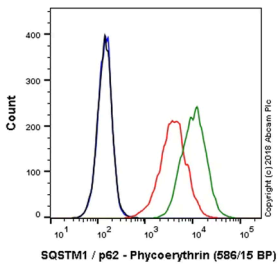
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109012).



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Flow Cytometry (Intracellular) staining using rabbit Anti-SQSTM1 / p62 antibody

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling SQSTM1 / p62 with purified ab109012 at 1/50 dilution (10µg/ml) (Red). Cells were fixed with 80% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109012).



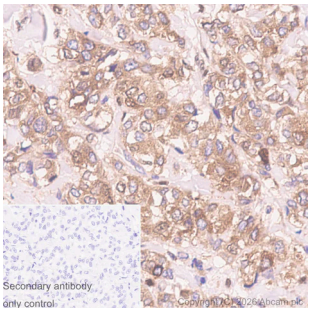
Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Flow Cytometry (Intracellular) staining using rabbit Anti-SQSTM1 / p62 antibody

Clone EPR4844 (ab219581) has been successfully conjugated by Abcam. This image was generated using Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (PE). Please refer to ab225094 for protocol details.
Overlay histogram showing HeLa cells untreated (red line) and HeLa cells treated with Chloroquine, 50µM, 24 hours, (green line) stained with ab225094. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab225094, 1/10000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (ab209478) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.



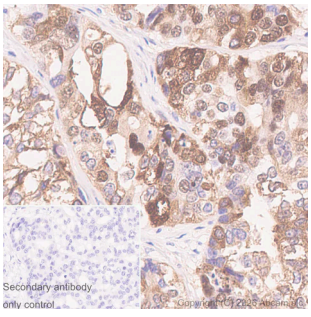
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human breast carcinoma using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labelling SQSTM1 / p62 with ab109012 at 1:10000 (0.044 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human breast carcinoma. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



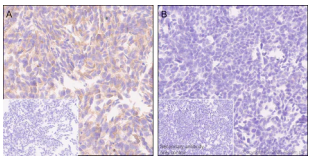
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human stomach carcinoma using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

Immunohistochemical analysis of paraffin-embedded Human stomach carcinoma tissue labelling SQSTM1 / p62 with ab109012 at 1:10000 (0.044 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human stomach carcinoma. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



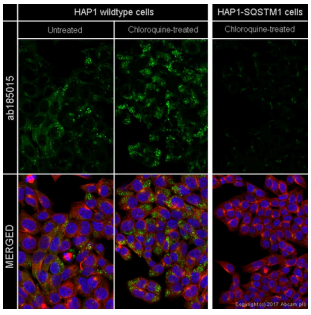
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

Immunohistochemical analysis of paraffin-embedded (A) Wild-type HCT116 (human colon epithelial) cell pellet and (B) SQSTM1 knockout HCT116 (ab266871) cell pellet labelling SQSTM1 / p62 with ab109012 at 1:10000 (0.044 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on (A) Wild-type HCT116 cell pellet, no staining on (B) SQSTM1 knockout HCT116 (ab266871) cell pellet. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.

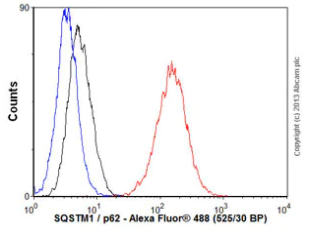


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SQSTM1 / p62 antibody

Clone EPR4844 (ab219581) has been successfully conjugated by Abcam. This image was generated using Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (Alexa Fluor® 488). Please refer to ab185015 for protocol details.
ab185015 staining SQSTM1 in wild-type HAP1 cells, untreated and chloroquine-treated (50µM, 24 hours) and chloroquine-treated SQSTM1 knockout HAP1 cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab185015 at 1/500 dilution (shown in green) and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

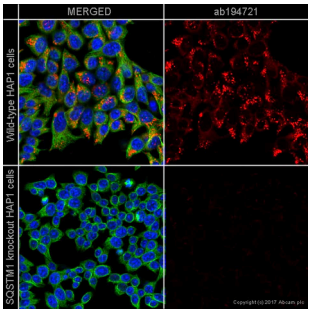


Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Flow Cytometry (Intracellular) staining using rabbit Anti-SQSTM1 / p62 antibody

Overlay histogram showing HeLa cells stained with unpurified ab109012 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab109012, 1/100 dilution) for 30 min at

22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109012).

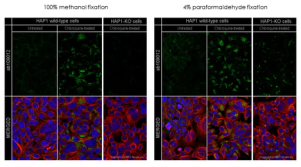


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SQSTM1 / p62 antibody

Clone EPR4844 (ab219581) has been successfully conjugated by Abcam. This image was generated using Anti-SQSTM1 / p62 antibody [EPR4844] (Alexa Fluor® 647). Please refer to ab194721 for protocol details.
ab194721 staining SQSTM1 in wild-type HAP1 cells (top panel) and SQSTM1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab194721 at 1/500 (shown in red) and ab195887 at 1/250 dilution (shown in green). Nuclear DNA was labelled in blue with DAPI.

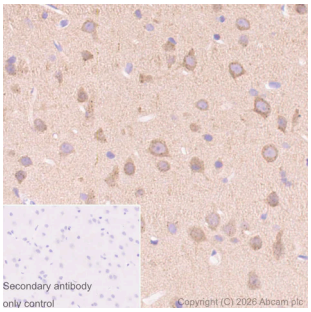
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab109012). ab109012 staining SQSTM1 in wild-type Hap1 cells and SQSTM1 knockout Hap1 cells treated with chloroquine (ab142116, 50µM for 24 hrs). The cells were fixed with 100% methanol (5 min) or with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109012 at 0.1µg/ml concentration and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).
Residual signal is observed in KO cells when paraformaldehyde is used for fixation, therefore we recommend using methanol fixation with this antibody. Alternatively, please use ab240635 or ab207305 which have been KO validated in both paraformaldehyde and methanol fixed cells.



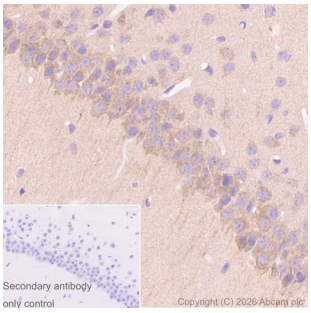
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat cerebrum using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labelling SQSTM1 / p62 with ab109012 at 1:10000 (0.044 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on rat cerebrum. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



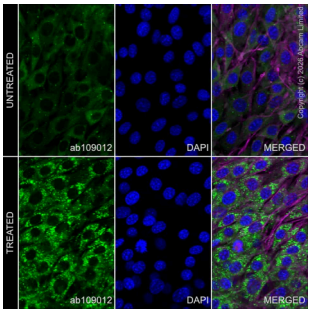
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse cerebrum using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labelling SQSTM1 / p62 with ab109012 at 1:10000 (0.044 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on mouse cerebrum. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

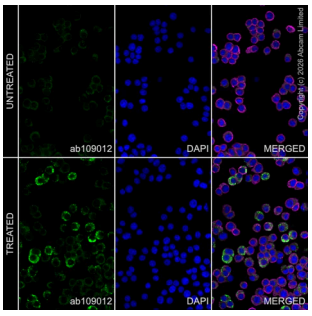
SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) labelling SQSTM1 / p62 with ab109012 at 1/500 dilution (0.874 µg/ml), followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution.

Confocal image showing increased cytoplasmic staining in NIH/3T3 cells treated with chloroquine (50uM) for 24 hr (shown in green). The primary counterstain, ab7291 Anti-alpha Tubulin mouse monoclonal antibody was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Secondary Counterstain: ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

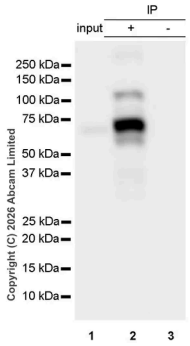
SQSTM1 / p62 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of PC-12 treated with chloroquine (50uM) for 24 hours using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized PC-12 (rat adrenal gland pheochromocytoma cell) labelling SQSTM1 / p62 with ab109012 at 1/200 dilution (2.5 µg/ml), followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution.

Confocal image showing increased cytoplasmic staining in PC-12 cells treated with chloroquine (50uM) for 24 hr (shown in green). The primary counterstain, ab7291 Anti-alpha Tubulin mouse monoclonal antibody was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Secondary Counterstain: ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor® 594)



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

SQSTM1 / p62 was immunoprecipitated from C6 (rat glial tumor glial cell) whole cell lysate 10 µg with ab109012 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab109012 at 1/1000 dilution.

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

All lanes:
Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/30 dilution

Lane 1:
C6 (rat glial tumor glial cell) whole cell lysate at 10 µg

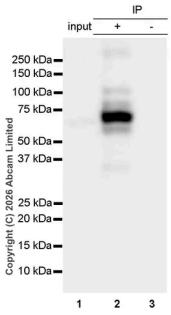
Lane 2:
C6 (rat glial tumor glial cell) whole cell lysate

Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab327347 in C6 whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 3s



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

This data was developed using the same antibody clone in a different formulation containing PBS and Azide (ab109012).

SQSTM1 / p62 was immunoprecipitated from MEF (mouse embryo fibroblast) whole cell lysate 10 µg with ab109012 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab109012 at 1/1000 dilution.

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

All lanes:
Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/30 dilution

Lane 1:
MEF (mouse embryo fibroblast) whole cell lysate at 10 µg

Lane 2:
MEF (mouse embryo fibroblast) whole cell lysate

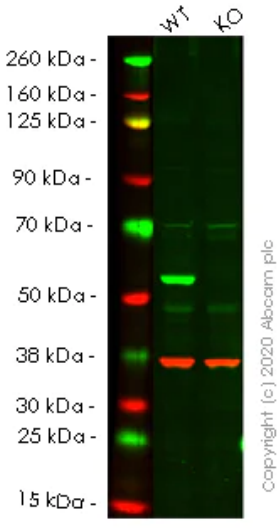
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab327347 in MEF whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 62 kDa

Exposure time: 3s



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Western blot staining using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab109012).
Lanes 1- 2: Merged signal (red and green). Green - ab109012 observed at 64 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab109012 was shown to react with SQSTM1/p62 in wild-type HEK293T cells in western blot. Loss of signal was observed when knockout cell line ab255343 (knockout cell lysate ab263770) was used. Wild-type HEK293T and SQSTM1 knockout HEK293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109012 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/10000 dilution

Lane 1:

Wild-type HEK293T cell lysate at 20 µg

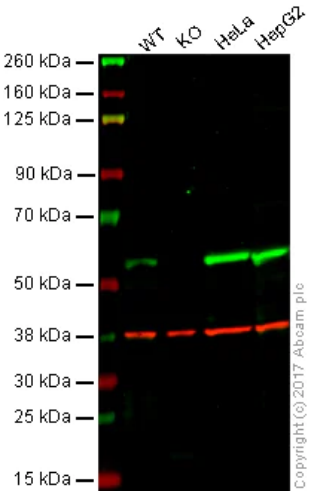
Lane 2:
SQSTM1 knockout HEK293T cell lysate at 20 µg

Lane 2:
Western blot - Human SQSTM1 (p62) knockout HEK-293T cell line (ab255343)

Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 64 kDa



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Western blot staining using rabbit Anti-SQSTM1 / p62 antibody

Lanes 1 - 4: Merged signal (red and green). Green - unpurifed ab109012 observed at 55 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab109012 was shown to specifically react with SQSTM1 in wild-type HAP1 cells. No band was observed when SQSTM1 knockout samples were used. Wild-type and SQSTM1 knockout samples were subjected to SDS-PAGE, ab109012 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/20,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109012).

All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/10000 dilution

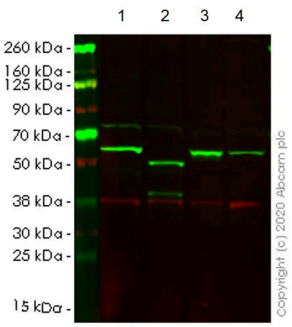
Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
SQSTM1 knockout HAP1 whole cell lysate at 20 µg

Lane 3:
HeLa whole cell lysate at 20 µg

Lane 4:
HepG2 whole cell lysate at 20 µg

Predicted band size: 47 kDa



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Western blot staining using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using ab109012, the same antibody clone in a different buffer formulation.
Lanes 1-4: Merged signal (red and green). Green - ab109012 observed at 55 kDa. Red - loading control ab8245 observed at 36 kDa.

ab109012 Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker was shown to specifically react with SQSTM1 / p62 in wild-type HCT116 cells. The band observed in CRISPR/Cas9 edited cell line ab266871 (CRISPR/Cas9 edited cell lysate ab257052) lane below 55 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and SQSTM1 / p62 CRISPR/Cas9 edited samples were subjected to SDS-PAGE. ab109012 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

Lane 1:
Wild-type HCT116 cell lysate at 20 µg

Lane 2:
SQSTM1 CRISPR/Cas9 edited HCT116 cell lysate at 20 µg

Lane 3:
HepG2 cell lysate at 20 µg

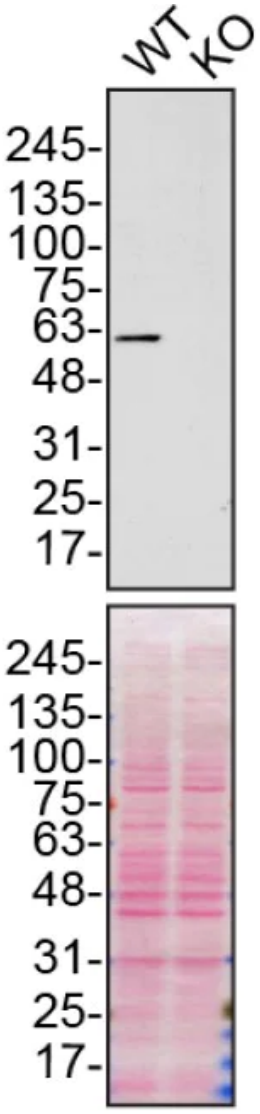
Lane 4:
HeLa cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 47 kDa

Observed band size: 55 kDa



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Western blot staining using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using the same antibody in a different buffer formulation (ab109012). ab109012 was shown to react with SQSTM1 in wild-type U-2 OS cells in Western blot with loss of signal observed in a SQSTM1 knockout cell line. Wild-type U-2 OS and SQSTM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab109012 overnight at 4 °C at a 1/10000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 0.2ug/mL before imaging. These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

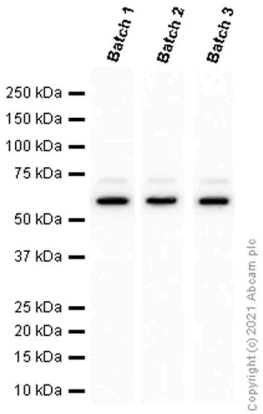
All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/10000 dilution

Lane 1:
Wild-type U-2 OS cell lysate at 20 µg

Lane 2:
SQSTM1 knockout U-2 OS cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 47 kDa



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - BSA and Azide free (ab219581)

SQSTM1 / p62 Western blot staining using rabbit Anti-SQSTM1 / p62 antibody

This data was developed using ab109012, the same antibody clone in a different buffer formulation. Different batches of ab109012 were tested on MCF7 (Human breast adenocarcinoma epithelial cell) lysate at 0.4 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 62 kDa.
All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

Predicted band size: 47 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.