abcam

ab109012

Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker

Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) is a rabbit monoclonal antibody detecting SQSTM1 / p62 in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, IHC-P**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 750 publications

Goat version of this clone also available as ab323241. Same clone, same staining pattern, different species antibody backbone.
Learn more about chimeric antibodies

Lab Essentials | Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
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	0.441 mg/mL The concentration of this product may be batch-dependent

Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Rat
Dilution info	1/10000
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	1/10000
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/10000
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Rat
Dilution info	1/200
Notes	-

Species	Mouse
Dilution info	1/500
Notes	-

Species	Human
Dilution info	1 µg/mL

Notes	-

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Species	Rat
Dilution info	1/30
Notes	-

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/10000 - 1/50000
Notes	-

Species	Rat
Dilution info	1/10000 - 1/50000
Notes	-

Species	Human
Dilution info	1/10000 - 1/50000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/100
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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Stable for 12 months at -20°C

Notes

Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IP and WB.

Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) was first used in a scientific publication in 2014 and has been cited over 445 times in peer reviewed journals. It's performance in Western Blot in human, mouse and rat samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) has been confirmed by Western Blot testing in SQSTM1 / p62 knockout HEK-293T cells (ab263770).

Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) has 15 independent reviews from customers.

Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) specifically detects SQSTM1 / p62 (UniProt ID: Q13501; Molecular weight: 48kDa) and is sold in 100 µL and 1 mL selling sizes.

Conjugation-ready, carrier free format available for antibody clone EPR4844 - ab219581.

Antibody clone EPR4844 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, HRP, Alexa Fluor® 647, Alexa Fluor® 594, Alexa Fluor® 555, APC, PE (ab185015, ab194720, ab194721, ab203429, ab203430, ab225093, ab225094).

NEW: Explore our alternative host species for clone [EPR4844] : goat. SQSTM1, also known as p62, is a multifunctional scaffolding protein widely used in neuro research for its role in autophagy and protein degradation. It is upregulated in several neurodegenerative disorders, including Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). Researchers use SQSTM1/p62 to study the mechanisms of protein aggregation and neuroinflammation, making it essential for understanding the progression of neurodegenerative diseases and identifying potential therapeutic targets.

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.

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

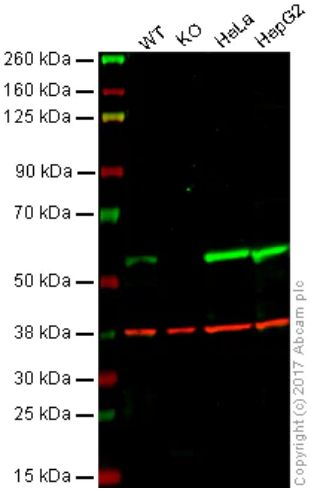
We do not recommend this combination. It is not covered by our product promise.

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.

25 product images



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

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

Lanes 1 - 4: Merged signal (red and green). Green - 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.

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

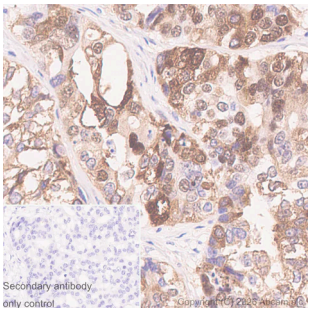
Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

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

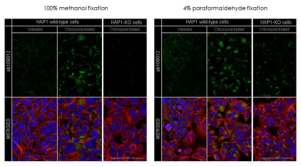


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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).

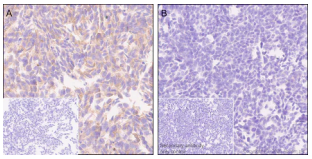
Positive staining on human stomach carcinoma. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20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



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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.

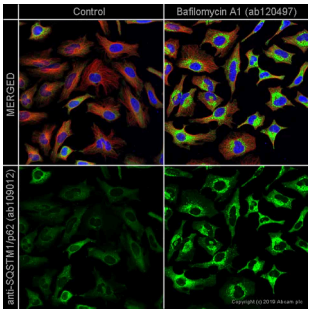


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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).

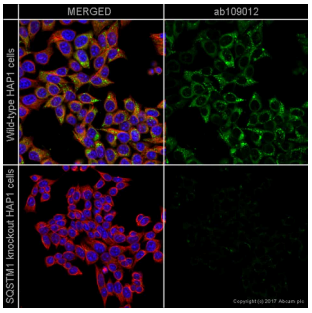
Positive staining on (A) Wild-type HCT116 cell pellet, no staining on (B) SQSTM1 knockout HCT116 (ab266871) cell pellet. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was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



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

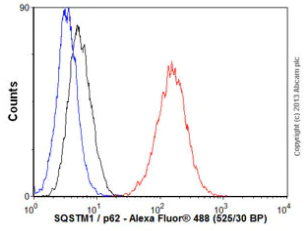
ab109012 staining SQSTM1/p62 (autophagosome) in control HeLa cells (left panel) and SQSTM1/p62 in HeLa cells treated with 1uM bafilomycin A1 (ab120497) for 18hrs (right panel). The cells were fixed with methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109012 at 5ug/ml and ab7291 (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 pseudo color red). 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] - Autophagosome Marker (ab109012)

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

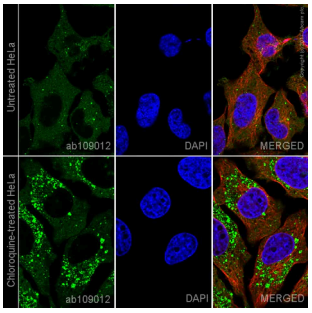
Purified ab109012 staining SQSTM1 in wild-type HAP1 cells (top panel) and SQSTM1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 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).



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

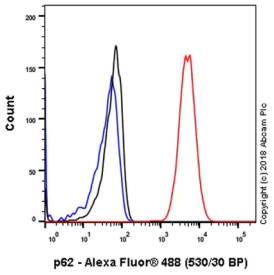
Overlay histogram showing HeLa cells stained with unpurifiedab109012 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab109012, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 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.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

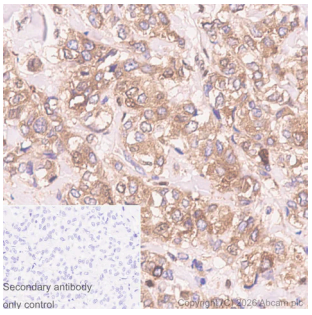
Unpurified ab109012 staining SQSTM1/p62 in HeLa cells +/- Chloroquine (50µM, 24 hours). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated 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).



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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 (10 µg/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).

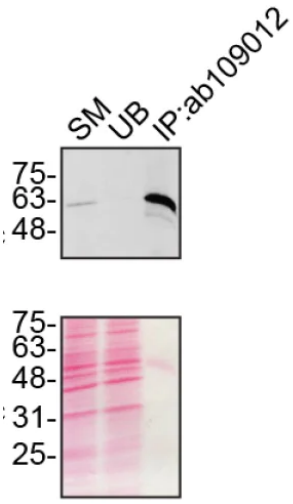


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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).

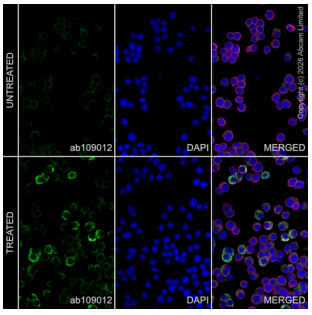
Positive staining on human breast carcinoma. The primary antibody was incubated for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20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



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

Immunoprecipitation of SQSTM1 in U-2 OS cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab109012 pre-coupled to prot.A-Sepharose beads. Samples were washed and processed for western blot with SQSTM1 / P62 antibody at 1/5000. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. 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:
Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

Predicted band size: 47 kDa



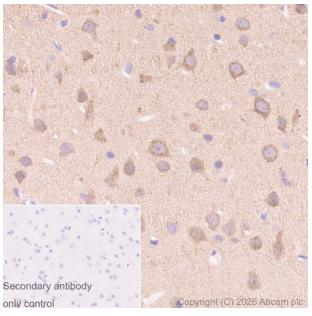
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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)

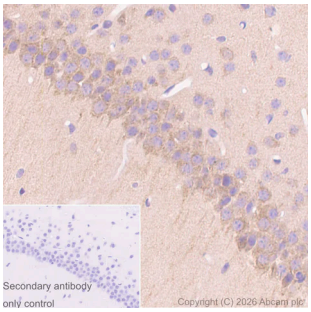


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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.

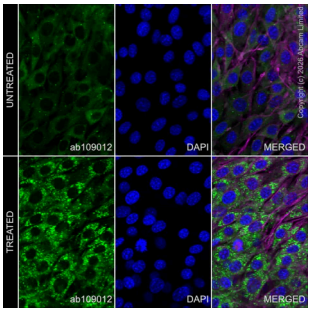


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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.



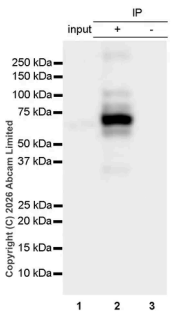
Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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.



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (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

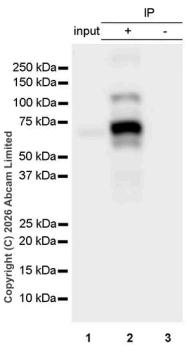
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab327347 in MEF whole cell lysate

Secondary

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

Observed band size: 62 kDa

Exposure time: 3s



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (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

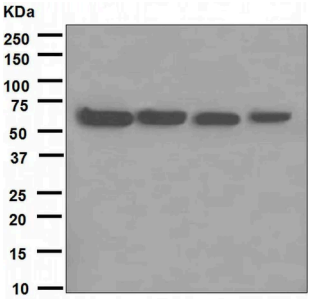
Lane 2:
C6 (rat glial tumor glial cell) whole cell lysate

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



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

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

Lane 1:
MCF-7 at 10 µg

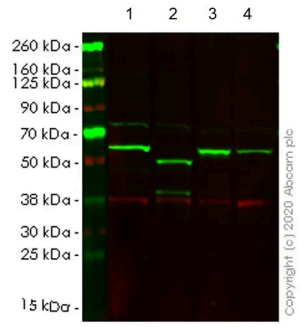
Lane 2:
HeLa at 10 µg

Lane 3:
SKBR-3 at 10 µg

Lane 4:
293T at 10 µg

Predicted band size: 47 kDa

Observed band size: 62 kDa



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

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

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 incubated overnight at 4°C at 1 in 1000 dilution 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

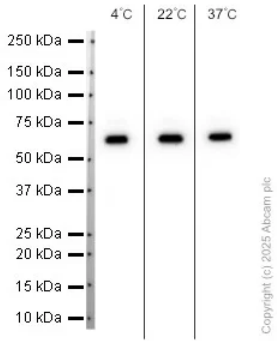
Secondary

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] - Autophagosome Marker (ab109012)

SQSTM1 / p62 Western blot staining of HeLa whole cell lysate using rabbit Anti-SQSTM1 / p62 antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of HeLa whole cell lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab109012 (1/1000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab109012 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

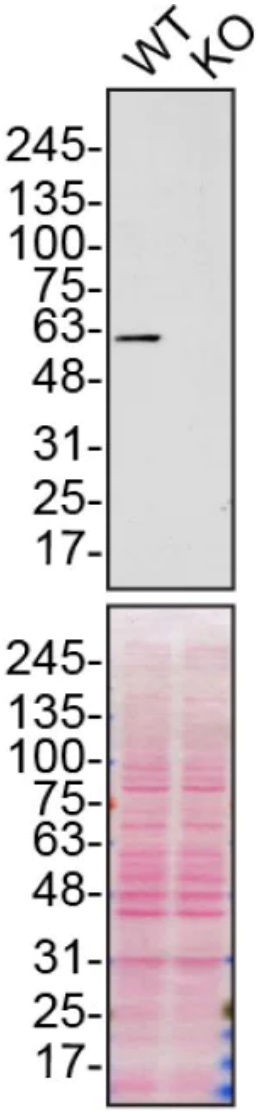
All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/1000 dilution

All lanes:
HeLa whole cell lysate at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Exposure time: 5s



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

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

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.

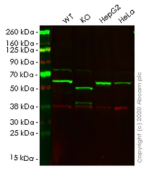
All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/10000 dilution

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] - Autophagosome Marker (ab109012)

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

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 knockout cell line ab266871 (knockout 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 knockout samples were subjected to SDS-PAGE. ab109012 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution 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 knockout HCT116 cell lysate at 20 µg

Lane 3:
HepG2 cell lysate at 20 µg

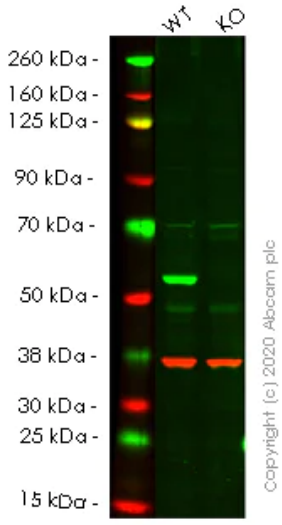
Lane 4:
HeLa cell lysate at 20 µg

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] - Autophagosome Marker (ab109012)

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

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

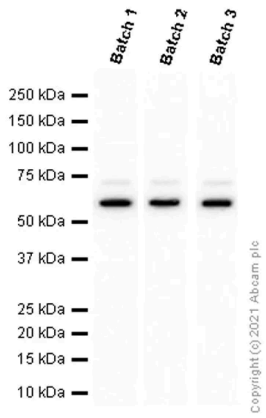
Lane 2:
SQSTM1 knockout HEK293T cell lysate at 20 µg

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

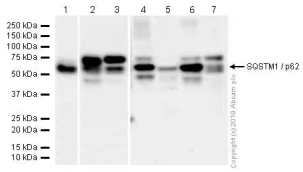


Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

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

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



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

All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/1000 dilution

Lane 1:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Lane 2:
Mouse brain lysates at 20 µg

Lane 3:
rat brain lysates at 20 µg

Lane 4:
Mouse lung lysates at 20 µg

Lane 5:
Rat lung lysates at 20 µg

Lane 6:
Mouse heart lysates at 20 µg

Lane 7:
Rat heart lysates at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 47 kDa

Observed band size: 62 kDa

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