abcam

ab227992

Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free

Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation. Suitable for **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF** in **Human**.

- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Biophysical QC for unrivalled batch-batch consistency

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	EPR18351
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	-

Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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

What is this antibody validated in?
Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of SQSTM1 / p62?
Anti-SQSTM1 / p62 [EPR18351] - BSA and Azide free (ab227992) specifically detects a band for SQSTM1 / p62 (UniProt: Q13501) at a molecular weight of 47kDa.

Specificity confirmed
The specificity of Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992) has been confirmed by Immunocytochemistry/ Immunofluorescence testing in SQSTM1 Knockout HAP1 cell line.

Other related products
We have a range of other formats of antibody clone [EPR18351] also available for your convenience: ab207305, Alexa Fluor® 647 - ab225453, PE - ab225454, Carrier free - ab227992

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.

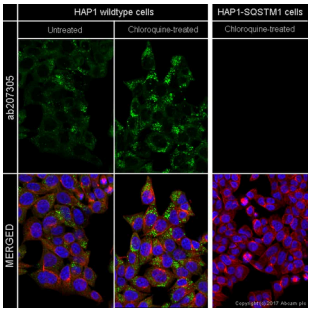
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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13 product images

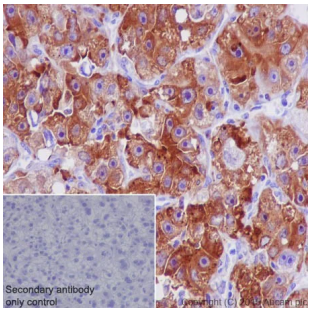


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

This ICC data was generated using the same anti-SQSTM1/p62 antibody clone, EPR18351, in a different buffer formulation (cat# ab207305).
ab207305 staining SQSTM1 in wild-type HAP1 cells and knockout cells, untreated and chloroquine-treated (50µM, 24 hours). 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 with ab207305 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).



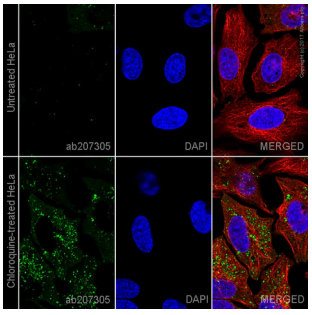
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

This IHC data was generated using the same anti-SQSTM1/p62 antibody clone, EPR18351, in a different buffer formulation (cat# ab207305).
Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma tissue labeling SQSTM1 / p62 with ab207305 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on human hepatocellular carcinoma is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

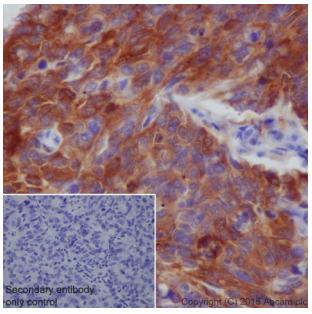


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

ab207305 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 ab207305 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 (ab207305).



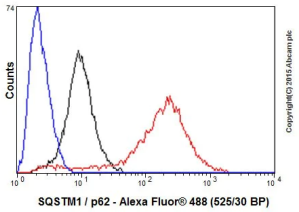
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling SQSTM1 / p62 with ab207305 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm and weakly nucleus staining on human lung cancer is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab207305).

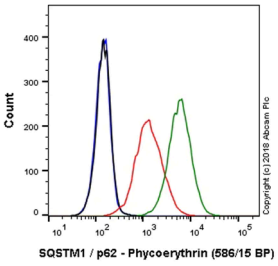
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed PC-3 (Human prostate adenocarcinoma cell line) cells labeling SQSTM1 / p62 with ab207305 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat Anti-Rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab207305).



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

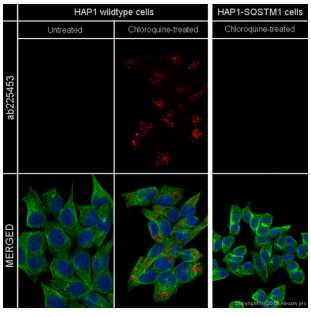
SQSTM1 / p62 Flow Cytometry (Intracellular) staining using rabbit Anti-SQSTM1 / p62 antibody

Clone EPR18351 (ab227992) has been successfully conjugated by Abcam. This image was generated using Anti-SQSTM1 / p62 antibody [EPR18351] (PE). Please refer to ab225454 for protocol details.
Overlay histogram showing HeLa cells untreated (red line) and HeLa cells treated with Chloroquine, 50µM, 24 hours, (green line) stained with ab225454. 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 (ab225454, 1/5000 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.

This antibody gave a positive signal in HeLa +/- Chloroquine cells fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



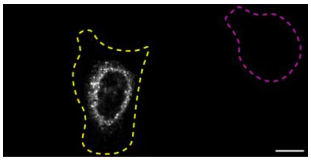
Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

Clone EPR18351 (ab227992) has been successfully conjugated by Abcam. This image was generated using Anti-SQSTM1 / p62 antibody [EPR18351] (Alexa Fluor® 647). Please refer to ab225453 for protocol details.
ab225453 staining SQSTM1 / p62 in wild-type HAP1 cells and knockout cells, untreated and chloroquine-treated (50µM, 24 hours). 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 with ab225453 at 5µg/ml (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

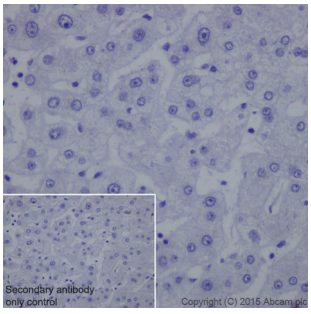
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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 (ab207305). ab207305 was shown to react with SQSTM1 in wild-type U-2 OS cells in Immunocytochemistry with loss of signal observed in a SQSTM1 knockout cell line. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1/5000. The cells were then incubated with ab207305 at 1/200 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880). 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.



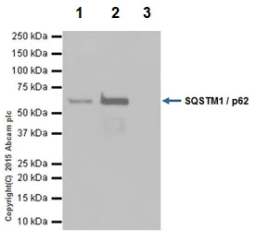
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling SQSTM1 / p62 with ab207305 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Negative staining on Hhman normal liver tissue. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab207305).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

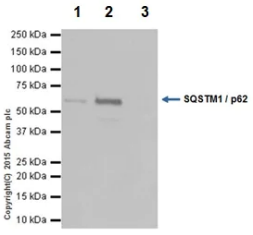


Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

SQSTM1 / p62 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab207305 at 1/40 dilution.
Western blot was performed from the immunoprecipitate using ab207305 at 1/1000 dilution.
VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: HeLa whole cell lysate 10ug (Input).
Lane 2: ab207305 IP in HeLa whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab207305 in HeLa whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 1 second.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab207305).

All lanes:
Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

Predicted band size: 47 kDa

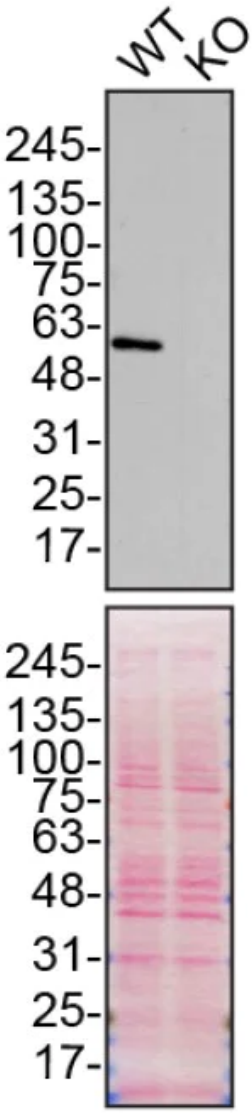


Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

SQSTM1 / p62 was immunoprecipitated from 1mg of HepG2 (Human liver hepatocellular carcinoma) whole cell lysate with ab207305 at 1/40 dilution.
Western blot was performed from the immunoprecipitate using ab207305 at 1/1000 dilution.
VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: HepG2 whole cell lysate 10ug (Input).
Lane 2: ab207305 IP in HepG2 whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab207305 in HepG2 whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 1 second.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab207305).

All lanes:
Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

Predicted band size: 32 kDa, 47 kDa



Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] - BSA and Azide free (ab227992)

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

This data was developed using the same antibody in a different buffer formulation (ab207305). ab207305 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 ab207305 overnight at 4 °C at a 1/1000 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.

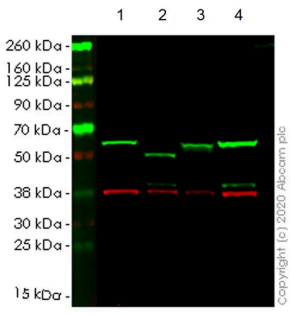
All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305) at 1/1000 dilution

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 [EPR18351] - BSA and Azide free (ab227992)

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

This data was developed using ab207305, the same antibody clone in a different buffer formulation.
Lanes 1-4: Merged signal (red and green). Green - ab207305 observed at 55 kDa. Red - loading control ab8245 observed at 36 kDa.

ab207305 Anti-SQSTM1 / p62 antibody [EPR18351] 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. ab207305 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 [EPR18351] - Autophagosome Marker (ab207305)

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

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