abcam

ab207305

Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker

Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305) is a rabbit monoclonal antibody detecting SQSTM1 / p62 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 40 publications

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	EPR18351
Purification technique	Affinity purification Protein A
Concentration	0.212 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/40
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	1/2000
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	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	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305) 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] (ab207305) specifically detects a band for SQSTM1 / p62 (UniProt: Q13501) at a molecular weight of 47kDa.

Trusted by the scientific community
Anti-SQSTM1 / p62 [EPR18351] (ab207305) was first used in a scientific publication in 2016 and has been cited over 40 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305) has been confirmed by Western blot testing in SQSTM1 Knockout HAP1 cells.

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our

selection of trial-size 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

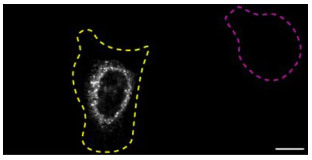
We do not recommend this combination. It is not covered by our product promise.

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.

18 product images

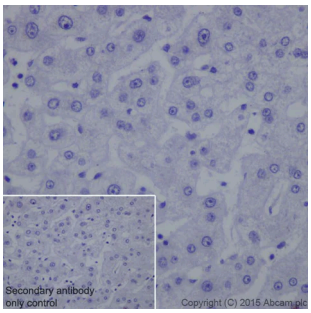


Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

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

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.

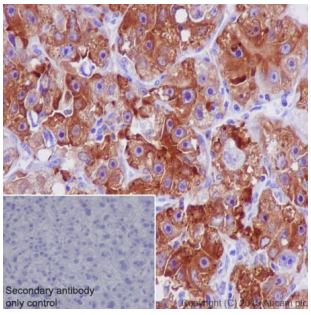


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

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.

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

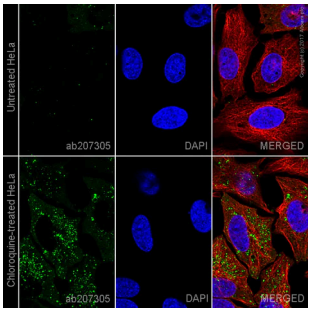


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

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

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.

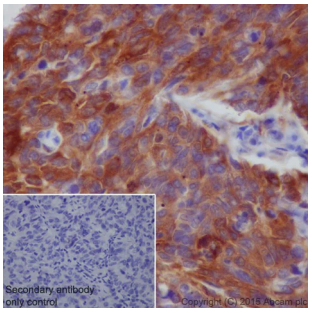
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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

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).

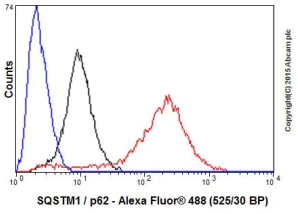


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

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.

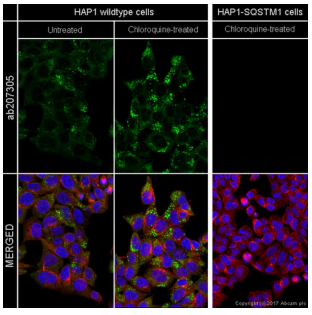
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

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

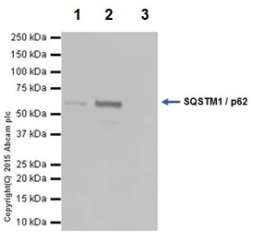
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed PC-3 (Human prostate adenocarcinoma cell line) cells labeling SQSTM1 / p62 with ab207305 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat Anti-Rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



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

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

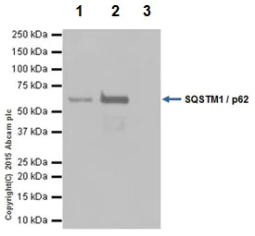
ab207305 staining SQSTM1 in wild-type HAP1 cells and knockout cells, untreated and chloroquine-treated (ab142116, 50µM, 24 hours). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% 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).



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

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

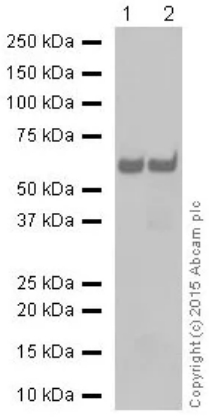
Predicted band size: 32 kDa, 47 kDa



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

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

Predicted band size: 47 kDa



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

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

Blocking/Dilution buffer: 5% NFDM/TBST.
The MW observed is consistent with what has been described in the literature (PMID: PMC4344198).

All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305) at 1/5000 dilution

Lane 1:
HepG2 (Human liver hepatocellular carcinoma) whole cell lysate at 10 µg

Lane 2:
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 10 µg

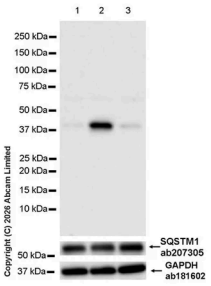
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 47 kDa

Observed band size: 62 kDa

Exposure time: 30s



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

SQSTM1 / p62 Western blot staining of HeLa treated with 10ng/ml TNF alpha and 1uM IKK-16 for 4 hours whole cell lysate using rabbit Anti-SQSTM1 / p62 antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

Total protein Anti-SQSTM1 antibody [EPR18351] (ab207305) (1:1000).

All lanes:
Western blot - Anti-cleaved SQSTM1 / p62 at D329 (N-terminal) antibody [EPR29289-46] (ab325681) at 1/1000 dilution

Lane 1:
Untreated HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
HeLa treated with 10ng/ml TNF alpha and 1uM IKK-16 for 4 hours whole cell lysate at 20 µg

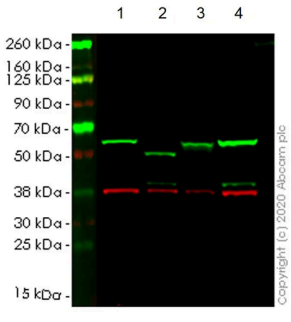
Lane 3:
HeLa treated with 10ng/ml TNF alpha, 1uM IKK-16 and 20uM Z-VAD-FMK for 4 hours whole cell lysate at 20 µg

Secondary

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

Observed band size: 40 kDa, 36 kDa

Exposure time: 10s



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

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

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

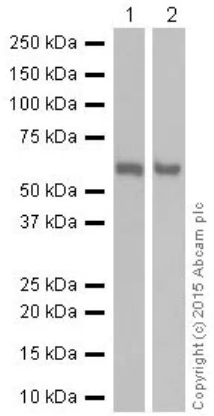
Lane 4:
HeLa cell lysate at 20 µg

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



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

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

Blocking/Dilution buffer: 5% NFDM/TBST.
The MW observed is consistent with what has been described in the literature (PMID: PMC4344198).

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

Lane 1:
Human liver lysate at 10 µg

Lane 2:
Human kidney lysate at 10 µg

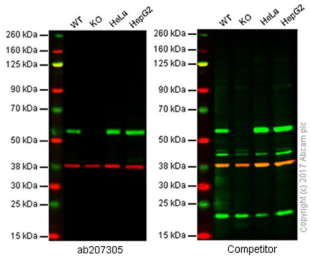
Secondary

All lanes:
Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 47 kDa

Observed band size: 62 kDa

Exposure time: 30s



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

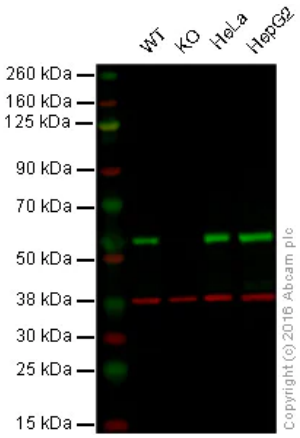
SQSTM1 / p62 Western blot staining using rabbit Anti-SQSTM1 / p62 antibody

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: SQSTM1/p62 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green).
Green - target observed at 55 kDa. Red - loading control, ab8245, observed at 37 kDa.

This western blot image is a comparison between ab207305 and a competitor's discontinued goat polyclonal antibody.

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

Predicted band size: 47 kDa



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

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

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: SQSTM1/p62 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab207305 observed at 55 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab207305 was shown to specifically react with SQSTM1/p62 in wild-type HAP1 cells. No band was observed when SQSTM1/p62 knockout samples were used. Wild-type and SQSTM1/p62 knockout samples were subjected to SDS-PAGE, ab207305 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/10,000 respectively and incubated overnight at 4°C. 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 1/10,000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] - Autophagosome Marker (ab207305)

Predicted band size: 47 kDa



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

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

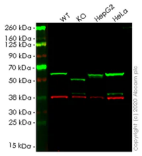
ab207305 was shown to react with SQSTM1 in wild-type U-2 OS cells in Western blot with loss of signal observed in a SQSTM1 knockout cell line. Wild-type U-2 OS and SQSTM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab207305 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] - Autophagosome Marker (ab207305)

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

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 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. 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 knockout HCT116 cell lysate at 20 µg

Lane 3:
HepG2 cell lysate at 20 µg

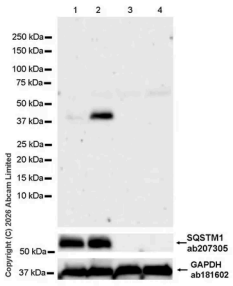
Lane 4:
HeLa cell lysate at 20 µg

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 [EPR18351] - Autophagosome Marker (ab207305)

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

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

In Western blot, ab325681 was shown to bind specifically to SQSTM1. Target of interest was observed at 40 kDa in TNF alpha and IKK-16 treated wild-type HAP1 cell lysates (lane 2) with no signal observed at this size in the SQSTM1 knockout cell line (lanes 3-4, knockout cell line ab240635).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

Total protein Anti-SQSTM1 antibody [EPR18351] (ab207305) (1:1000).

All lanes:
Western blot - Anti-cleaved SQSTM1 / p62 at D329 (N-terminal) antibody [EPR29289-46] (ab325681) at 1/1000 dilution

Lane 1:
Untreated parental HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate at 60 µg

Lane 2:
HAP1 treated with 10ng/ml TNF alpha and 1uM IKK-16 for 4 hours whole cell lysate at 60 µg

Lane 3:
Untreated SQSTM1 knockout HAP1 whole cell lysate at 60 µg

Lane 4:
SQSTM1 knockout HAP1 treated with 10ng/ml TNF alpha and 1uM IKK-16 for 4 hours whole cell lysate at 60 µg

Secondary

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

Observed band size: 40 kDa, 36 kDa

Exposure time: 180s

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