abcam

ab211324

Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451]

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	EPR20451
Purification technique	Affinity purification Protein A
Concentration	0.515 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

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

Target data

See full target information SQSTM1 pS349

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

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.

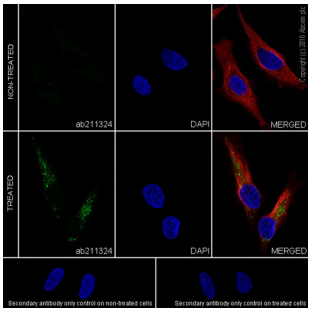
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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7 product images



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324)

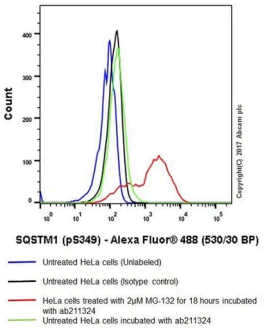
SQSTM1 / p62 (phospho S349) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SQSTM1 / p62 (phospho S349) antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 2µM MG-132 (ab141003) for 18 hours or untreated, labeling SQSTM1 / p62 (phospho S349) with ab211324 at 1/100 dilution, followed by Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) secondary antibody at 1/200 dilution (green).
Confocal image showing cytoplasmic staining on HeLa cell line. The expression increased after treatment with 2µM MG-132 (ab141003) for 18 hours.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

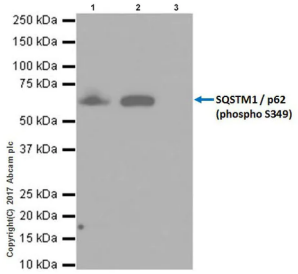
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324)

SQSTM1 / p62 (phospho S349) Flow Cytometry (Intracellular) staining using rabbit Anti-SQSTM1 / p62 (phospho S349) antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 2µM MG-132 (ab141003) for 18 hours (red) or untreated (green), labeling SQSTM1 / p62 (phospho S349) with ab211324 at 1/500 dilution compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324)

SQSTM1 / p62 (phospho S349) was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 2µM MG-132 (ab141003) for 18h with ab211324 at 1/30 dilution.
Western blot was performed from the immunoprecipitate using ab211324 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.

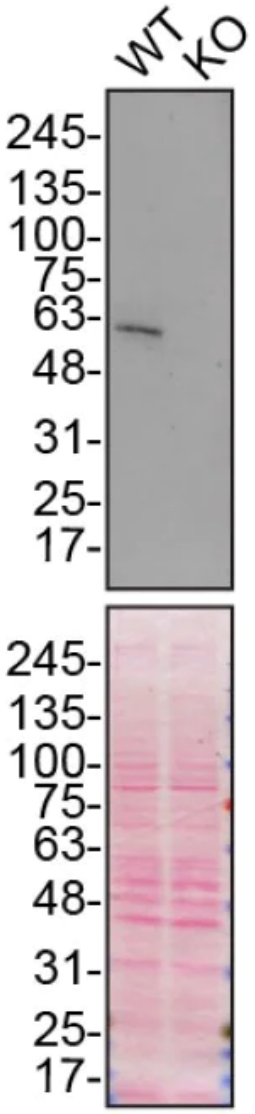
Lane 1: HeLa treated with 2µM MG-132 (ab141003) for 18h whole cell lysate, 10 µg (Input).
Lane 2: ab211324 IP in HeLa treated with 2µM MG-132 (ab141003) for 18h whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab211324 in HeLa treated with 2µM MG-132 (ab141003) for 18h whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324)

Predicted band size: 47 kDa

Exposure time: 10s



Western blot - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324)

SQSTM1 / p62 (phospho S349) Western blot staining using rabbit Anti-SQSTM1 / p62 (phospho S349) antibody

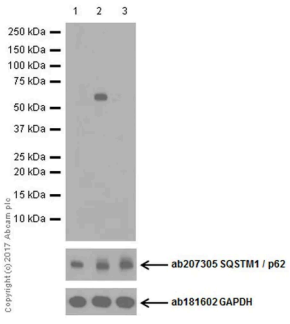
ab211324 was shown to react with SQSTM1 in wild-type U-2 OS cells in Western blot with loss of signal observed in a SQSTM1 knockout cell line. Wild-type U-2 OS and SQSTM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab211324 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 (phospho S349) antibody [EPR20451] (ab211324) 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 (phospho S349) antibody [EPR20451] (ab211324)

SQSTM1 / p62 (phospho S349) Western blot staining using rabbit Anti-SQSTM1 / p62 (phospho S349) antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324) at 1/1000 dilution

Lane 1:
Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

Lane 2:
HeLa whole cell lysate treated with 2µM MG-132 (ab141003) for 18 hours at 10 µg

Lane 3:
HeLa whole cell lysate treated with 2µM MG-132 (ab141003) for 18 hours, then treated with Alkaline Phosphatase for 1 hour at 10 µg

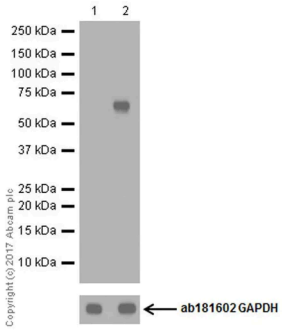
Secondary

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: 1min



Western blot - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324) at 1/1000 dilution

Lane 1:
Untreated C6 (Rat glial tumor cell line) whole cell lysate at 10 µg

Lane 2:
C6 whole cell lysate treated with 2µM MG-132 (ab141003) for 18 hours at 10 µg

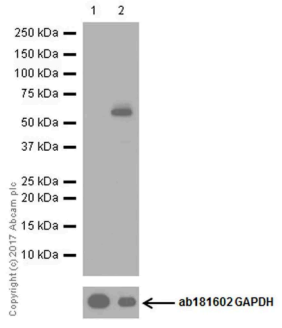
Secondary

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

Predicted band size: 34 kDa, 47 kDa, 76 kDa

Observed band size: 42 kDa, 62 kDa, 76 kDa

Exposure time: 1min



Western blot - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324) at 1/1000 dilution

Lane 1:
Untreated NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate at 10 µg

Lane 2:
NIH/3T3 whole cell lysate treated with 2µM MG-132 (ab141003) for 18 hours 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: 4s

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