abcam

ab185015

Alexa Fluor® 488 Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Conjugation	Alexa Fluor® 488
Excitation/Emission	Ex: 495nm, Em: 519nm
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% 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
Concentration	0.5 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/500
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	ab199091 - Rabbit monoclonal IgG (Alexa FluorÂ® 488), is suitable for use as an isotype control with this antibody.

Expected

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

Target data

See full target information SQSTM1

Function Molecular adapter required for selective macroautophagy (aggrephagy) by acting as a bridge between polyubiquitinated proteins and autophagosomes (PubMed:15340068, PubMed:15953362, PubMed:16286508, PubMed:17580304, PubMed:20168092, PubMed:22017874, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:29343546, PubMed:29507397, PubMed:31857589, PubMed:33509017, PubMed:34471133, PubMed:34893540, PubMed:35831301, PubMed:37306101, PubMed:37802024). Promotes the recruitment of ubiquitinated cargo proteins to autophagosomes via multiple domains that bridge proteins and organelles in different steps (PubMed:16286508, PubMed:20168092, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:29343546, PubMed:29507397, PubMed:34893540, PubMed:37802024). SQSTM1 first mediates

the assembly and removal of ubiquitinated proteins by undergoing liquid-liquid phase separation upon binding to ubiquitinated proteins via its UBA domain, leading to the formation of insoluble cytoplasmic inclusions, known as p62 bodies (PubMed:15911346, PubMed:20168092, PubMed:22017874, PubMed:24128730, PubMed:29343546, PubMed:29507397, PubMed:31857589, PubMed:37802024). SQSTM1 then interacts with ATG8 family proteins on autophagosomes via its LIR motif, leading to p62 body recruitment to autophagosomes, followed by autophagic clearance of ubiquitinated proteins (PubMed:16286508, PubMed:17580304, PubMed:20168092, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:37802024). SQSTM1 is itself degraded along with its ubiquitinated cargos (PubMed:16286508, PubMed:17580304, PubMed:37802024). Also required to recruit ubiquitinated proteins to PML bodies in the nucleus (PubMed:20168092). Also involved in autophagy of peroxisomes (pexophagy) in response to reactive oxygen species (ROS) by acting as a bridge between ubiquitinated PEX5 receptor and autophagosomes (PubMed:26344566). Acts as an activator of the NFE2L2/NRF2 pathway via interaction with KEAP1: interaction inactivates the BCR(KEAP1) complex by sequestering the complex in inclusion bodies, promoting nuclear accumulation of NFE2L2/NRF2 and subsequent expression of cytoprotective genes (PubMed:20452972, PubMed:28380357, PubMed:33393215, PubMed:37306101). Promotes relocalization of 'Lys-63'-linked ubiquitinated STING1 to autophagosomes (PubMed:29496741). Involved in endosome organization by retaining vesicles in the perinuclear cloud: following ubiquitination by RNF26, attracts specific vesicle-associated adapters, forming a molecular bridge that restrains cognate vesicles in the perinuclear region and organizes the endosomal pathway for efficient cargo transport (PubMed:27368102, PubMed:33472082). Sequesters tensin TNS2 into cytoplasmic puncta, promoting TNS2 ubiquitination and proteasomal degradation (PubMed:25101860). May regulate the activation of NFKB1 by TNF, nerve growth factor (NGF) and interleukin-1 (PubMed:10356400, PubMed:10747026, PubMed:11244088, PubMed:12471037, PubMed:16079148, PubMed:19931284). May play a role in titin/TTN downstream signaling in muscle cells (PubMed:15802564). Adapter that mediates the interaction between TRAF6 and CYLD (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage 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, Stable for 12 months at -20°C, Store in the dark

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.

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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

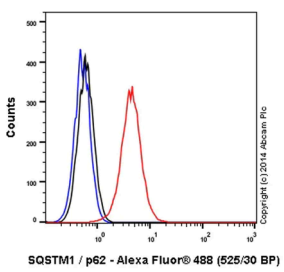
We do not recommend this combination. It is not covered by our product promise.

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.

4 product images

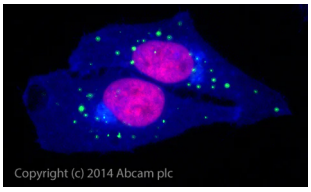


Flow Cytometry (Intracellular) - Alexa Fluor® 488 Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab185015)

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

Overlay histogram showing HeLa cells stained with ab185015 (red line). The cells were fixed with 4% formaldehyde (10 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 (ab185015, 1/500 dilution) for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 488 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 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

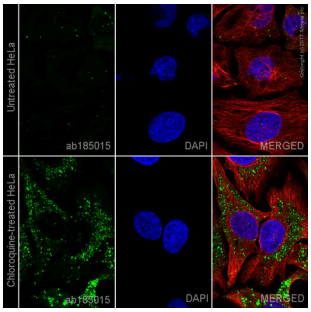
This antibody gave a positive signal in HeLa fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab185015)

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

ab185015 staining SQSTM1/p62 in HeLa cells. The cells were fixed with 4% PFA (10min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab185015 (Alexa Fluor® 488) at a working dilution of 1 in 100 overnight at +4°C (shown in green). AlexaFluor® 350 WGA was used at a 1/200 dilution and incubated for 1h with the cells, to label plasma membranes (shown in blue). Nuclear DNA was labelled in red with 1.25 µM DRAQ5™ (ab108410).

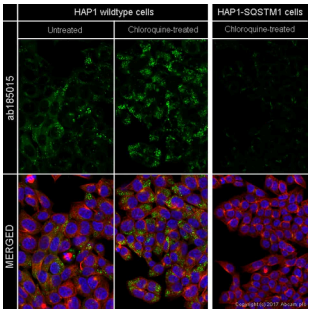


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab185015)

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

ab185015 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 ab185015 at 1/500 dilution (shown in green) and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

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



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab185015)

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

ab185015 staining SQSTM1 in wild-type HAP1 cells, untreated and chloroquine-treated (50µM, 24 hours) and chloroquine-treated SQSTM1 knockout HAP1 cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab185015 at 1/500 dilution (shown in green) and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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