abcam

Product datasheet

Anti-SQSTM1 / p62 antibody [EPR23101-103] ab240635

KO VALIDATED Recombinant RabMAb

9 References 13 Images

Overview

Product name	Anti-SQSTM1 / p62 antibody [EPR23101-103]
Description	Rabbit monoclonal [EPR23101-103] to SQSTM1 / p62
Host species	Rabbit
Specificity	WB: This antibody is not suitable for using in rat tissues. We suggest loading 40µg lysate per lane in gel to obtain good signal.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IP **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: U-2 OS, HeLa, MEF, NIH/3T3, PC-12, C2C12 and J774A.1 lysates. ICC/IF: HeLa, U-2 OS and MEF cells. Flow Cyt (intra): HeLa and MEF cells. IP: HeLa, U-2 OS and MEF cells.
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR23101-103
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab240635 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 62 kDa (predicted molecular weight: 47 kDa).
ICC/IF		Use a concentration of 1 µg/ml.
IP		1/30.

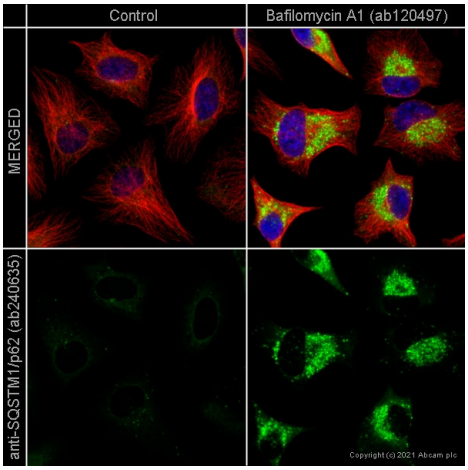
Application notes Is unsuitable for IHC-P.

Target

Function	Adapter protein which binds ubiquitin and may regulate the activation of NFKB1 by TNF-alpha, nerve growth factor (NGF) and interleukin-1. May play a role in titin/TTN downstream signaling in muscle cells. May regulate signaling cascades through ubiquitination. Adapter that mediates the interaction between TRAF6 and CYLD (By similarity). May be involved in cell differentiation, apoptosis, immune response and regulation of K(+) channels.
Tissue specificity	Ubiquitously expressed.
Involvement in disease	Defects in SQSTM1 are a cause of Paget disease of bone (PDB) [MIM:602080]. PDB is a metabolic bone disease affecting the axial skeleton and characterized by focal areas of increased and disorganized bone turn-over due to activated osteoclasts. Manifestations of the disease include bone pain, deformity, pathological fractures, deafness, neurological complications and increased risk of osteosarcoma. PDB is a chronic disease affecting 2 to 3% of the population above the age of 40 years.
Sequence similarities	Contains 1 OPR domain. Contains 1 UBA domain. Contains 1 ZZ-type zinc finger.
Domain	The UBA domain binds specifically 'Lys-63'-linked polyubiquitin chains of polyubiquitinated substrates. Mediates the interaction with TRIM55. The OPR domain mediates homooligomerization and interactions with PRKCZ, PRKCI, MAP2K5 and NBR1. The ZZ-type zinc finger mediates the interaction with RIPK1.
Post-translational modifications	Phosphorylated. May be phosphorylated by PRKCZ (By similarity). Phosphorylated in vitro by TTN.
Cellular localization	Cytoplasm. Late endosome. Nucleus. Sarcomere (By similarity). In cardiac muscles localizes to

the sarcomeric band (By similarity). Localizes to late endosomes. May also localize to the nucleus. Accumulates in neurofibrillary tangles and in Lewy bodies of neurons from individuals with Alzheimer and Parkinson disease respectively. Enriched in Rosenthal fibers of pilocytic astrocytoma. In liver cells, accumulates in Mallory bodies associated with alcoholic hepatitis, Wilson disease, indian childhood cirrhosis and in hyaline bodies associated with hepatocellular carcinoma.

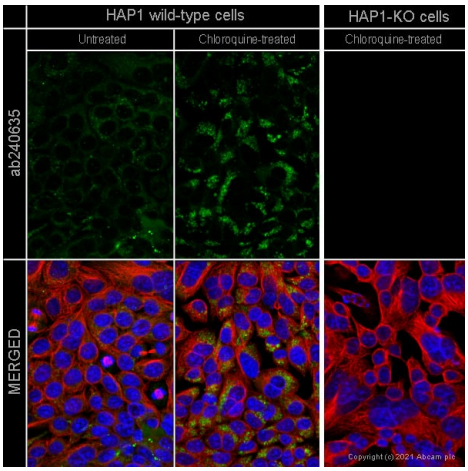
Images



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

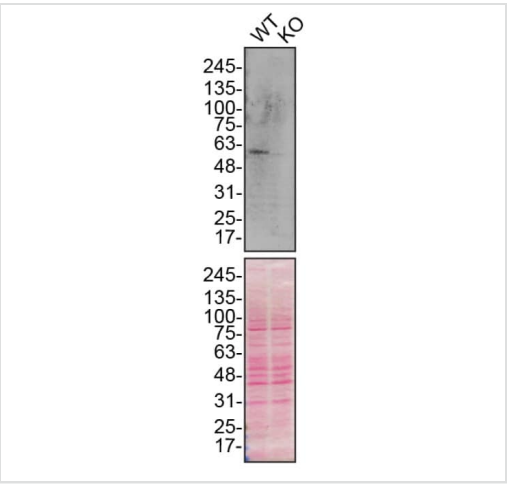
ab240635 staining SQSTM1/p62 (autophagosome) in control HeLa cells (left panel) and SQSTM1/p62 in HeLa cells treated with 100nM bafilomycin A1 (**ab120497**) for 18hrs (right panel). The cells were fixed with methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab240635 at 2ug/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 red). Nuclear DNA was labelled in blue with DAPI.

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

ab240635 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) 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 ab240635 at 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).



Western blot - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

All lanes : Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635) at 1/1000 dilution

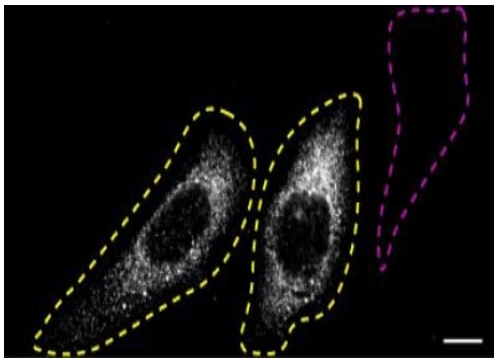
Lane 1 : Wild-type U-2 OS cell lysate
Lane 2 : SQSTM1 knockout U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa

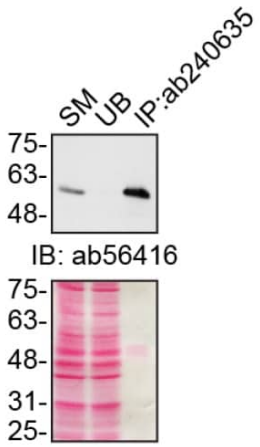
ab240635 was shown to react with SQSTM1 in wild-type U-2 OS cells in Western blot with loss of signal observed in a SQSTM1 knockout cell line. Wild-type U-2 OS and SQSTM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab240635 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.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

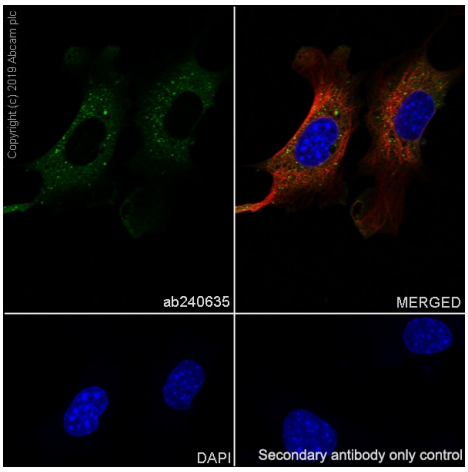
ab240635 was shown to react with SQSTM1 in wild-type U-2 OS cells in Immunocytochemistry with loss of signal observed in a SQSTM1 knockout cell line. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1/5000. The cells were then incubated with ab240635 at 1/500 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.



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

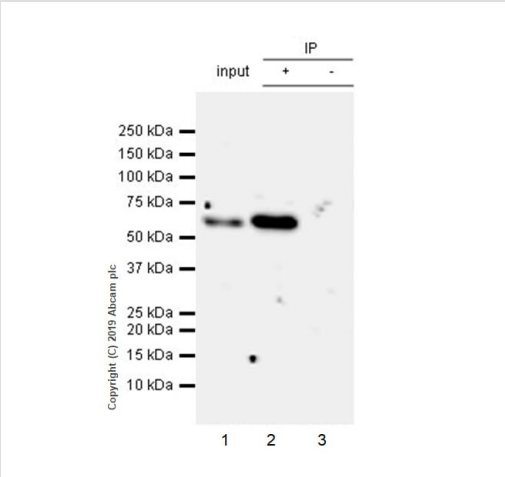
Immunoprecipitation of SQSTM1 in U-2 OS cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab240635 pre-coupled to prot.A-Sepharose beads. Samples were washed and processed for western blot with **ab56416** at 1/5000. SM=10% starting material; UB=10% unbound fraction; 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.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized MEF (Mouse embryo fibroblast) cells labelling SQSTM1 / p62 with ab240635 at 1/50 dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in MEF cells. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution.



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

SQSTM1 / p62 was immunoprecipitated from 0.35 mg MEF (Mouse embryonic fibroblast (immortalized)) whole cell lysate with ab240635 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab240635 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

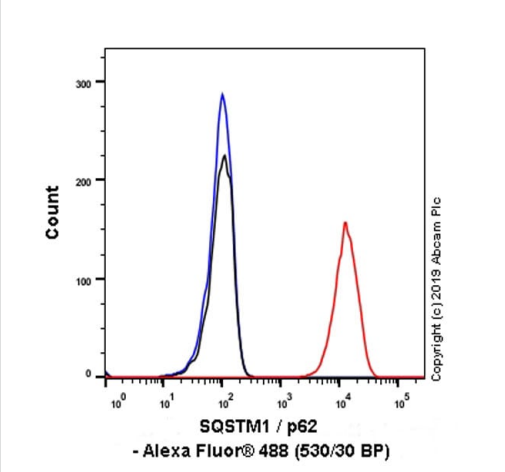
Lane 1: MEF whole cell lysate 10ug

Lane 2: ab240635 IP in MEF whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab240635 in MEF whole cell lysate

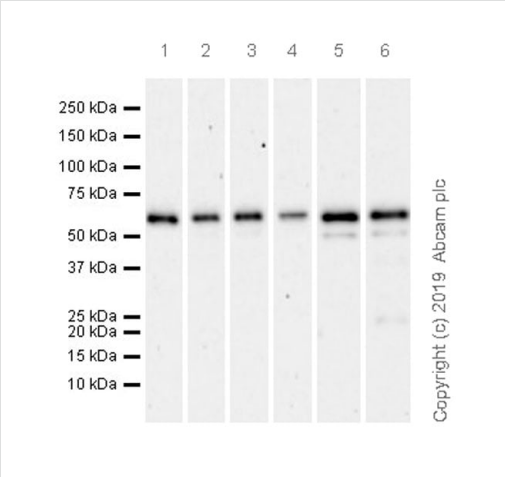
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 min.



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized MEF (Mouse embryonic fibroblast (immortalized)) cells labelling SQSTM1 / p62 with ab240635 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

All lanes : Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 2 : MEF (mouse embryonic fibroblast (immortalized)), whole cell lysate
Lane 3 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate
Lane 4 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate
Lane 5 : C2C12 (mouse myoblasts myoblast), whole cell lysate
Lane 6 : J774A.1 (mouse reticulum cell sarcoma monocyte macrophage), whole cell lysate

Lysates/proteins at 20 µg per lane.

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

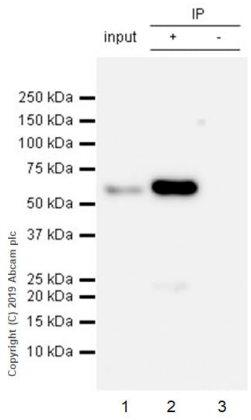
Predicted band size: 47 kDa
Observed band size: 62 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times.

Lanes 1-4:3 minutes; Lanes 5-6:92 seconds.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 24086455).



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SQSTM1 / p62 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with ab240635 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab240635 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

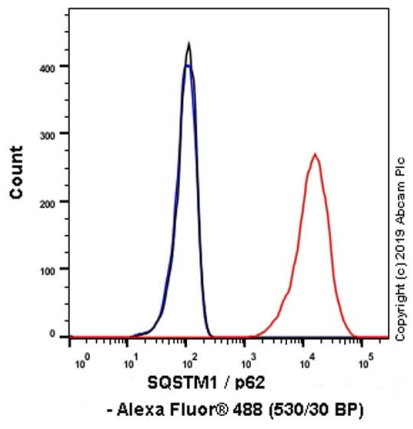
Lane 1: HeLa whole cell lysate 10ug

Lane 2: ab240635 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab240635 in HeLa whole cell lysate

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

Exposure time: 30 seconds.



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

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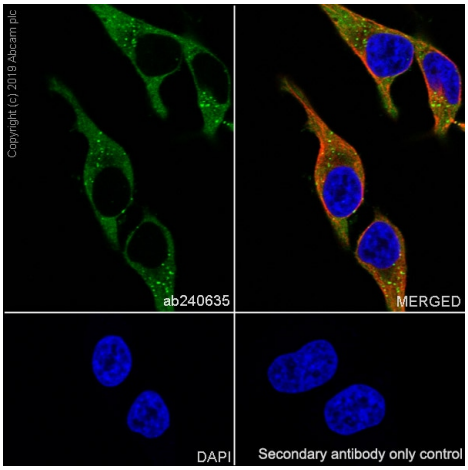

Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling SQSTM1 / p62 with ab240635 at 1/50 dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HeLa cells. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

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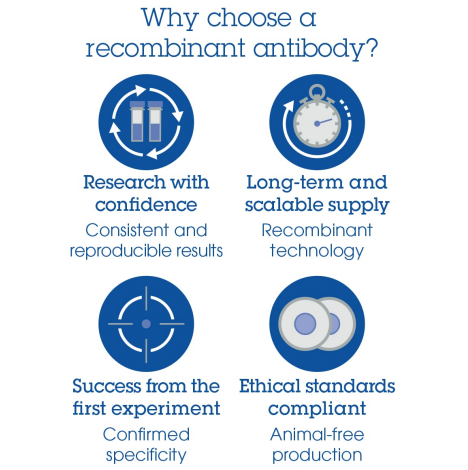

Anti-SQSTM1 / p62 antibody [EPR23101-103] (ab240635)

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