abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451]
Description	Rabbit monoclonal [EPR20451] to SQSTM1 / p62 (phospho S349)
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: U-2 OS, HeLa, C6 and NIH/3T3 whole cell lysates treated with 2ÂµM MG-132 (**ab141003**) for 18 hours. ICC/IF: HeLa cells treated with 2ÂµM MG-132 (**ab141003**) for 18 hours. Flow Cyt (intra): HeLa cells treated with 2ÂµM MG-132 (**ab141003**) for 18 hours. IP: HeLa treated with 2ÂµM MG-132 (**ab141003**) for 18h whole cell lysate.
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, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR20451
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab211324 in the following tested applications.

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

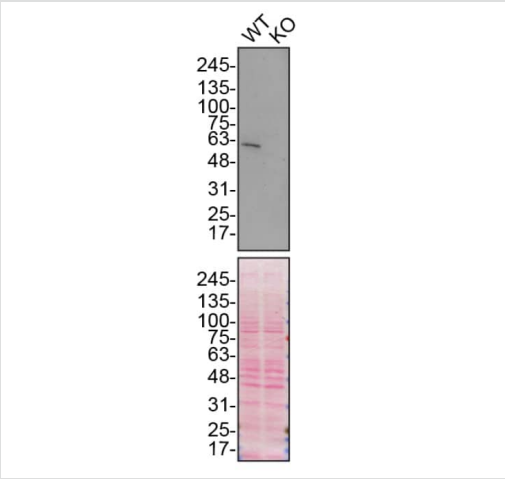
Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
ICC/IF	★★★★★ (1)	1/100.
IP		1/30.
WB	★★★★★ (2)	1/1000. Detects a band of approximately 62 kDa (predicted molecular weight: 47 kDa).

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



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

All lanes : Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324) at 1/1000 dilution

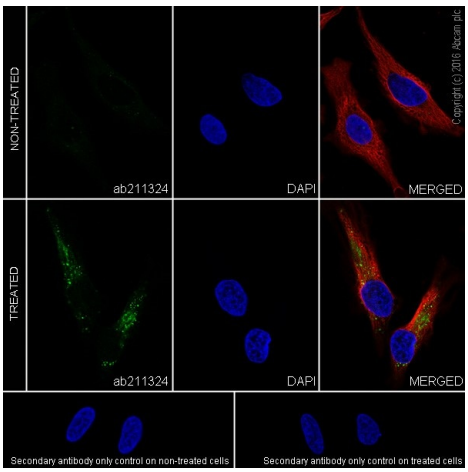
Lane 1 : Wild-type U-2 OS cell lysate
Lane 2 : SQSTM1 knockout U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa

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.



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

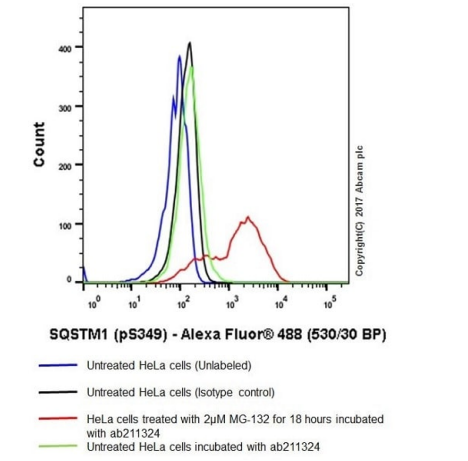
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 2µM MG-132 (**ab141003**) for 18 hours or untreated, labeling SQSTM1 / p62 (phospho S349) with ab211324 at 1/100 dilution, followed 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).

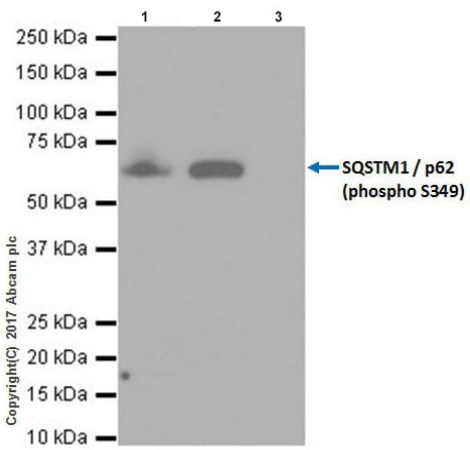
Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

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)

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)

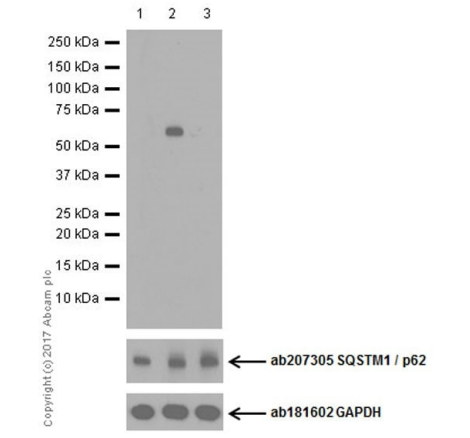
SQSTM1 / p62 (phospho S349) was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 2µM MG-132 (**ab141003**) for 18h with ab211324 at 1/30 dilution.

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.



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

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

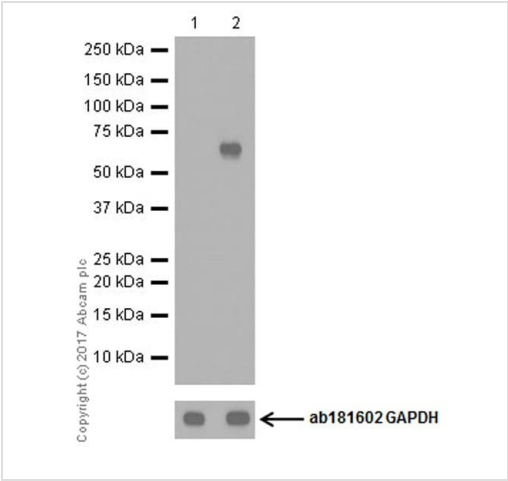
Lysates/proteins at 10 µg per lane.

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

Exposure time: 1 minute

Blocking/dilution buffer: 5% NFDM/TBST.



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

All lanes : Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] (ab211324) at 1/1000 dilution

Lane 1 : Untreated C6 (Rat glial tumor cell line) whole cell lysate
Lane 2 : C6 whole cell lysate treated with 2µM MG-132 (**ab141003**) for 18 hours

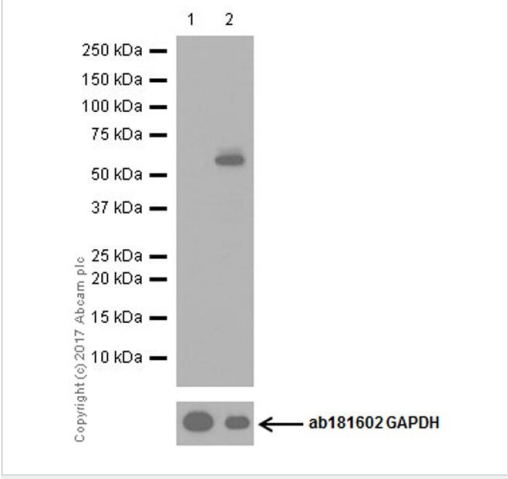
Lysates/proteins at 10 µg per lane.

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

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



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

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

Lysates/proteins at 10 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

Exposure time: 4 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

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

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