abcam

Product datasheet

Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] - BSA and Azide free ab238445







5 Images

Overview

Product name Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] - BSA and Azide free

Description Rabbit monoclonal [EPR20451] to SQSTM1 / p62 (phospho S349) - BSA and Azide free

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 ICC/IF: HeLa cells treated with 2µM MG-132 for 18 hours. WB: U-2 OS cell lysate.

General notes ab238445 is the carrier-free version of ab211324.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR20451

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab238445 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) | | Use at an assay dependent concentration. |
| ICC/IF | ★★★★ (1) | Use at an assay dependent concentration. |
| IP | | Use at an assay dependent concentration. |
| WB | ★★★ ☆ ☆ (1) | Use at an assay dependent concentration. 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.

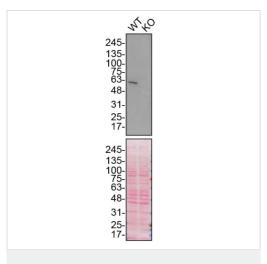
Post-translational modifications

Cellular localization

Phosphorylated. May be phosphorylated by PRKCZ (By similarity). Phosphorylated in vitro by TTN.

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] - BSA and Azide free (ab238445) **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

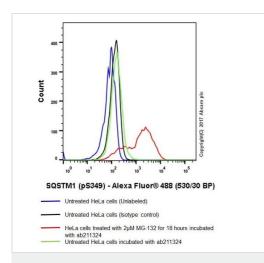
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

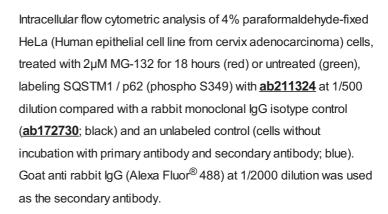
Predicted band size: 47 kDa

This data was developed using the same antibody in a different buffer formulation (ab211324).

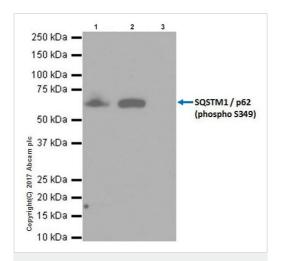
ab211324 was shown to react with SQSTM1 in wild-type U-2 OS cells in Western blot with loss of signal observed in a SQSTM1 knockout cell line. Wild-type U-2 OS and SQSTM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab211324 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat antirabbit 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.



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] - BSA and Azide free (ab238445)



This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab211324**).



Immunoprecipitation - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] - BSA and Azide free (ab238445) SQSTM1 / p62 (phospho S349) was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 2 μ M MG-132 for 18h with <u>ab211324</u> at 1/30 dilution.

Western blot was performed from the immunoprecipitate using **ab211324** at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: HeLa treated with $2\mu M$ MG-132 for 18h whole cell lysate, 10 μg (Input).

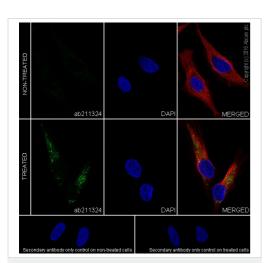
Lane 2: <u>ab211324</u> IP in HeLa treated with $2\mu M$ MG-132 for 18h whole cell lysate.

Lane 3: Rabbit monoclonal IgG ($\underline{ab172730}$) instead of $\underline{ab211324}$ in HeLa treated with 2 μ M MG-132 for 18h whole cell lysate.

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

Exposure time: 10 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab211324</u>).



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 (phospho S349) antibody [EPR20451] - BSA and Azide free (ab238445) 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 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 $2\mu M$ MG-132 for 18 hours.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab211324).



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