Anti-SQSTM1 / p62 antibody ab56416

Overview

Product name: Anti-SQSTM1 / p62 antibody
Description: Mouse monoclonal to SQSTM1 / p62
Host species: Mouse
Tested applications: Suitable for: IHC-P, WB, ICC/IF, Flow Cyt, IHC-Fr
Species reactivity: Reacts with: Mouse, Rat, Human, Rhesus monkey, Chinese hamster
Immunogen: Recombinant full length protein, corresponding to amino acids 1-441 of Human SQSTM1/p62
General notes: Abcam recommended secondaries - Goat Anti-Mouse HRP (ab205719) and Goat Anti-Mouse Alexa Fluor® 488 (ab150113).
See other anti-mouse secondary antibodies that can be used with this antibody.

Properties

Form: Liquid
Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer: Preservative: None PBS, pH 7.2
Purity: Protein G purified
Clonality: Monoclonal
Isotype: IgG2a
Light chain type: kappa

Applications

Our Abpromise guarantee covers the use of ab56416 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
</table>
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.

Application | Abreviews | Notes
--- | --- | ---
IHC-P | 🟣🟢🟢🟢🟢 | Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB | 🟣🟢🟢🟢🟢 | Use a concentration of 1 - 5 µg/ml.
ICC/IF | 🟣🟢🟢🟢🟢 | Use a concentration of 10 µg/ml. Fix cells for 15 minutes with 2 mL of 4% paraformaldehyde solution (pH 7.4 with NaOH in PBS). Permeabilize cells by incubating for 15 minutes on ice with 2 mL of 0.1% Triton X-100 in PBS.
Flow Cyt | 🟣🟢🟢🟢🟢 | Use 0.5µg for 10⁶ cells. 
ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody. 
We recommend Goat Anti-Mouse IgG H&L (DyLight® 488) preadsorbed (ab96879) secondary antibody
IHC-Fr | 🟣🟢🟢🟢🟢 | Use at an assay dependent concentration. PubMed: 22577215
Western blot - Anti-SQSTM1 / p62 antibody (ab56416)

This image is courtesy of an Abreview submitted by Melanie Thelen.

**All lanes**: Anti-SQSTM1 / p62 antibody (ab56416) at 1/2000 dilution

**All lanes**: HEK293 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: HRP-conjugated goat anti-mouse IgG at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Observed band size**: 61 kDa

why is the actual band size different from the predicted?

**Exposure time**: 10 seconds

Blocked with 5% milk for 3 hours at 21°C.

Incubated with the primary antibody for 17 hours at 4°C.

Monoclonal antibody to SQSTM1 (ab56416) on HeLa cell, antibody concentration 10 µg/ml.
ab56416 staining SQSTM1/p62 in Human A431 epidermoid cancer cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with formaldehyde, permeabilized with Triton X-100 and blocked with 5% BSA for 30 minutes at room temperature. Samples were incubated with primary antibody (1/50) in 5% BSA for 1 hour. An Alexa Fluor® 488-conjugated Goat monoclonal to mouse IgG (1/50) was used as secondary antibody.

Overlay histogram showing HeLa cells stained with ab56416 (red line). The cells were fixed with 80% methanol (5 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 (ab56416, 0.5µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was Goat Anti-Mouse IgG H&L (DyLight® 488) preadsorbed (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody (ab56416)

ab56416 (1µg/ml) staining SQSTM1 in human lymph node using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear and cytoplasmic staining.

Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH 6.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.

All lanes: Anti-SQSTM1 / p62 antibody (ab56416) at 1/1000 dilution

Lane 1: Control
Lane 2: Starved in HBSS for 2 hours
Lane 3: Starved in HBSS for 4 hours
Lane 4: Starved in HBSS for 8 hours
Lane 5: Starved in HBSS 8 hours + 200 nM Baf A1
Lane 6: Starved in HBSS 8 hours + 4 uM Mg132

Lysates/proteins at 40 µg per lane.

Secondary

All lanes: HRP conjugated goat anti-mouse polyclonal at 1/5000 dilution

Developed using the ECL technique.

Observed band size: 62 kDa why is the actual band size different from the predicted?

Exposure time: 10 seconds

All lanes are whole cell lysate prepared from HeLa cells. Treatments are listed.
All lanes: Anti-SQSTM1 / p62 antibody (ab56416) at 1/1000 dilution

Lane 1: Whole cell lysates prepared from Tzb-naive SKBR3 parental cells.

Lane 2: Whole cell lysates prepared from Tzb-refractory TzbR POOL1 cells.

Lane 3: Whole cell lysates prepared from Tzb-refractory TzbR POOL2 cells.

Lysates/proteins at 50 µg per lane.

Secondary

All lanes: Horseradish peroxidase-conjugated secondary

Developed using the ECL technique.

Cells were washed twice with cold-PBS and then lysed in buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton® X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na3VO4, 1 µg/mL leupeptin, 1 mM phenylmethylsulfonylfluoride, and complete protease inhibitor cocktail for 30 minutes on ice. The lysates were cleared by centrifugation in an Eppendorff tube (15 minutes at 14,000×g, 4°C). Protein content was determined against a standardized control using the Pierce Protein Assay Kit. Equal amounts of protein were resuspended in 5× Laemmli sample buffer (10 minutes at 70°C), resolved by electrophoresis on 10% SDS-PAGE, and transferred onto nitrocellulose membranes. Non-specific binding on the nitrocellulose filter paper was minimized by blocking for 1 hour at room temperature with TBS-T buffer [25 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Tween 20] containing 5% (w/v) nonfat dry milk. The treated filters were washed in TBS-T and then incubated with the primary

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
• We provide support in Chinese, English, French, German, Japanese and Spanish
• Extensive multi-media technical resources to help you
• We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors