abcam

## Product datasheet

# Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free ab56416

KO VALIDATED

36 Abreviews 731 References 7 Images

### Overview

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|---|---|
| **Product name** | Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free |
| **Description** | Mouse monoclonal [2C11] to SQSTM1 / p62 - BSA and Azide free |
| **Host species** | Mouse |
| **Tested applications** | **Suitable for:** IHC-P, IP, WB, ICC/IF, Flow Cyt |
| **Species reactivity** | **Reacts with:** Human |
| **Immunogen** | Recombinant full length protein corresponding to Human SQSTM1/ p62 aa 1-440.<br>Database link: Q13501 |
| **Positive control** | WB: HeLa, Hap1, U-2 OS cell lysates. Flow Cyt: HeLa. IHC-P: Human lymph node. ICC/IF: HeLa and U-2 OS cells. IP: U-2 OS cell lysate |
| **General notes** | This product was changed from ascites to tissue culture supernatant on 28th May 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.<br><br>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.<br><br>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As |

### Properties

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| **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** | pH: 7.40<br>Constituent: PBS |
| **Carrier free** | Yes |

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| **Purity** | Tissue culture supernatant |
| **Clonality** | Monoclonal |
| **Clone number** | 2C11 |
| **Isotype** | IgG2a |
| **Light chain type** | kappa |

## Applications

**The Abpromise guarantee** 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.

| Application | Abreviews | Notes |
|---|---|---|
| **IHC-P** | ★★★★★ (3) | Use at an assay dependent concentration.<br>Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |
| **IP** | ★★★★★ (1) | Use at an assay dependent concentration. |
| **WB** | ★★★★★ (23) | Use at an assay dependent concentration. |
| **ICC/IF** | ★★★★★ (4) | Use at an assay dependent concentration.<br>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 at an assay dependent concentration.<br>ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.<br>We recommend Goat Anti-Mouse IgG H&L (DyLight® 488) preadsorbed (ab96879) secondary antibody |

## 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.

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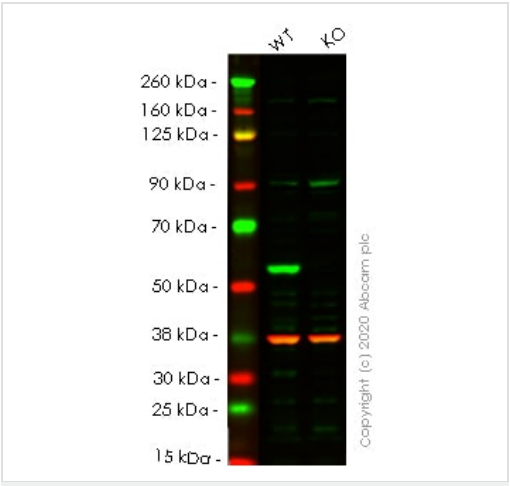
| **Sequence similarities** | Contains 1 OPR domain.<br>Contains 1 UBA domain.<br>Contains 1 ZZ-type zinc finger. |
| **Domain** | The UBA domain binds specifically 'Lys-63'-linked polyubiquitin chains of polyubiquitinated substrates. Mediates the interaction with TRIM55.<br>The OPR domain mediates homooligomerization and interactions with PRKCZ, PRKCI, MAP2K5 and NBR1.<br>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 antibody [2C11] - BSA and Azide free (ab56416)

**All lanes :** Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416) at 1/1000 dilution

**Lane 1 :** Wild-type HEK293T cell lysate
**Lane 2 :** SQSTM1 knockout HEK293T cell lysate

Lysates/proteins at 20 µg per lane.

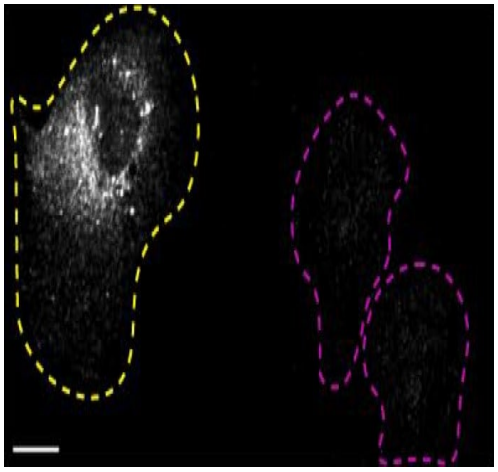
Performed under reducing conditions.

**Observed band size:** 62 kDa

**Lanes 1- 2:** Merged signal (red and green). Green - ab56416 observed at 62 kDa. Red - Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) observed at 37 kDa.

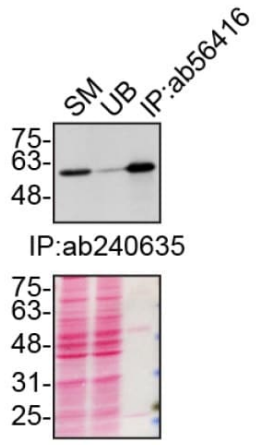
ab56416 was shown to react with SQSTM1/p62 in wild-type HEK293T cells in western blot. Loss of signal was observed when knockout cell line ab255343 (knockout cell lysate ab263770) was used. Wild-type HEK293T and SQSTM1 knockout HEK293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab56416 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) overnight at 4°C at a 1 in 1000 dilution and a 1

in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye®800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye®680RD) preadsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



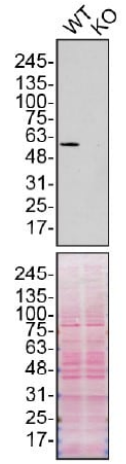
Immunocytochemistry - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

ab56416 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 1/10 000. The cells were then incubated with ab56416 at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-mouse 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.ing knock out cell lines.




Immunoprecipitation - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

Immunoprecipitation of SQSTM1 in U-2 OS cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab56416 pre-coupled to prot.G-Sepharose beads. Samples were washed and processed for western blot with ab207305 at 1/10,000. 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.




Western blot - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

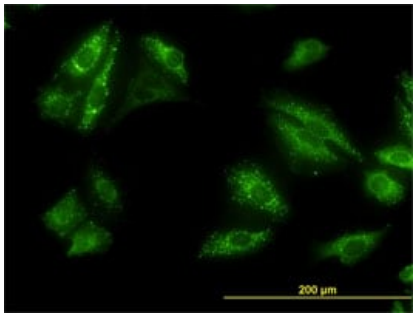
**All lanes :** Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416) at 1/1000 dilution

**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.

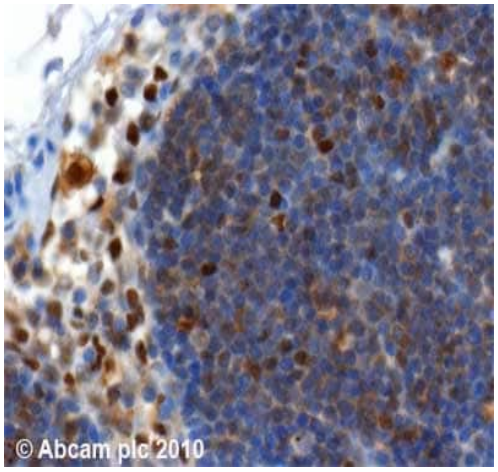
ab56416 was shown to react with SQSTM1 in wild-type U-2 OS cells in Western blot with loss of signal observed in a SQSTM1 knockout cell line. Wild-type U-2 OS and SQSTM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab56416 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-mouse HRP secondary antibodies at 0.3ug/mL before imaging. This data was kindly provided by the YCharOS Inc., an open science company with the mission of characterizing every commercially available antibody reagent. Abcam are working with YCharOS to support their mission of antibody characterisation using knockout cell lines.




Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

Monoclonal antibody to SQSTM1 (ab56416) on HeLa cell, antibody concentration 10 ug/ml.

This image was generated using the ascites version of the product.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (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.

This image was generated using the ascites version of the product.

Flow Cytometry - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

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/1x$10^6$ cells) for 30 min at 22ºC. The secondary antibody used was Goat Anti-Mouse IgG H&L (DyLight® 488) preadsorbed (ab96879) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.

This image was generated using the ascites version of the product.



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