abcam

ab56416

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

Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416) is a mouse monoclonal antibody provided in a PBS only buffer for easy conjugation detecting SQSTM1 / p62 in **Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF**. Suitable for **Human**.

- Clone 2C11 is the most cited clone to SQSTM1 / p62
- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Over 1040 publications

KO Validated

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	pH: 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Full Length Protein corresponding to Human SQSTM1. Database link Q13501
Carrier free	Yes
Clone number	2C11
Purity	Tissue culture supernatant
Light chain type	kappa

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab91361 or ab18415 - 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

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Human

Dilution info	-
Notes	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.

Target data

See full target information SQSTM1

Function	Molecular adapter required for selective macroautophagy (aggrephagy) by acting as a bridge between polyubiquitinated proteins and autophagosomes (PubMed:15340068, PubMed:15953362, PubMed:16286508, PubMed:17580304, PubMed:20168092, PubMed:22017874, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:29343546, PubMed:29507397, PubMed:31857589, PubMed:33509017, PubMed:34471133, PubMed:34893540, PubMed:35831301, PubMed:37306101, PubMed:37802024). Promotes the recruitment of ubiquitinated cargo proteins to autophagosomes via multiple domains that bridge proteins and organelles in different steps (PubMed:16286508, PubMed:20168092, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:29343546, PubMed:29507397, PubMed:34893540, PubMed:37802024). SQSTM1 first mediates the assembly and removal of ubiquitinated proteins by undergoing liquid-liquid phase separation upon binding to ubiquitinated proteins via its UBA domain, leading to the formation of insoluble cytoplasmic inclusions, known as p62 bodies (PubMed:15911346, PubMed:20168092, PubMed:22017874, PubMed:24128730, PubMed:29343546, PubMed:29507397, PubMed:31857589, PubMed:37802024). SQSTM1 then interacts with ATG8 family proteins on autophagosomes via its LIR motif, leading to p62 body recruitment to autophagosomes, followed by autophagic clearance of ubiquitinated proteins (PubMed:16286508, PubMed:17580304, PubMed:20168092, PubMed:22622177, PubMed:24128730, PubMed:28404643, PubMed:37802024). SQSTM1 is itself degraded along with its ubiquitinated cargos (PubMed:16286508, PubMed:17580304, PubMed:37802024). Also required to recruit ubiquitinated proteins to PML bodies in the nucleus (PubMed:20168092). Also involved in autophagy of peroxisomes (pexophagy) in response to reactive oxygen species (ROS) by acting as a bridge between ubiquitinated PEX5 receptor and autophagosomes (PubMed:26344566). Acts as an activator of the NFE2L2/NRF2 pathway via interaction with KEAP1: interaction inactivates the BCR(KEAP1) complex by sequestering the complex in inclusion bodies, promoting nuclear accumulation of NFE2L2/NRF2 and subsequent expression of cytoprotective genes (PubMed:20452972, PubMed:28380357, PubMed:33393215, PubMed:37306101). Promotes relocalization of 'Lys-63'-linked ubiquitinated STING1 to autophagosomes (PubMed:29496741). Involved in endosome organization by retaining vesicles in the perinuclear cloud: following ubiquitination by RNF26, attracts specific vesicle-associated adapters, forming a molecular bridge that restrains cognate vesicles in the perinuclear region and organizes the endosomal pathway for efficient cargo transport (PubMed:27368102, PubMed:33472082). Sequesters tensin TNS2 into cytoplasmic puncta, promoting TNS2 ubiquitination and proteasomal degradation (PubMed:25101860). May regulate the activation of NFKB1 by TNF, nerve growth factor (NGF) and interleukin-1 (PubMed:10356400, PubMed:10747026, PubMed:11244088, PubMed:12471037, PubMed:16079148, PubMed:19931284). May play a role in titin/TTN downstream signaling in muscle cells

(PubMed:15802564). Adapter that mediates the interaction between TRAF6 and CYLD (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Product Specifications
Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416) is a mouse monoclonal antibody provided in a carrier-free, conjugation ready format.
ab56416 is validated in Flow Cyt, ICC/IF, IHC-P, IP, WB in human samples.
Carrier-free antibodies are compatible with our best-in-class range of Lightning-Link® conjugation kits.
Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416) specifically detects SQSTM1 / p62 (UniProt ID: Q13501; Molecular weight: 48kDa) and is sold in 100 ug selling sizes.

Quality and Validation
Antibody clone 2C11 was first used in a scientific publication in 1976 and has been cited over 1046 times in peer reviewed journals and is trusted by the scientific community.
Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416) has 36 independent reviews from customers.
The specificity of Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416) has been confirmed by testing in knockout samples.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

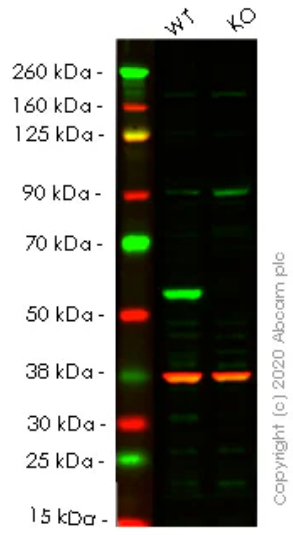
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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7 product images



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

SQSTM1 / p62 Western blot staining using mouse Anti-SQSTM1 / p62 antibody

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.

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

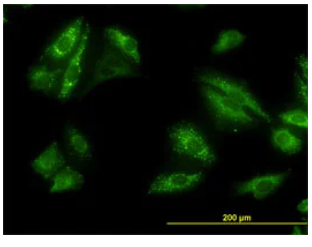
Lane 1:
Wild-type HEK293T cell lysate at 20 µg

Lane 2:
Western blot - Human SQSTM1 (p62) knockout HEK-293T cell lysate (ab263770) at 20 µg

Performed under reducing conditions.

Predicted band size: 47 kDa

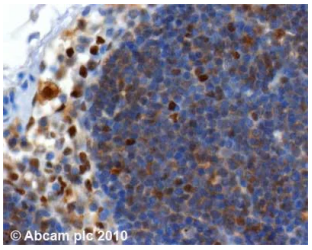
Observed band size: 62 kDa



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

SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-SQSTM1 / p62 antibody

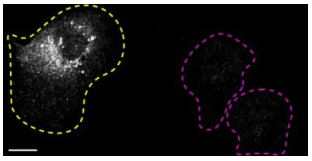
Monoclonal antibody to SQSTM1 (ab56416) on HeLa cell, antibody concentration 10 ug/ml.
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

SQSTM1 / p62 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-SQSTM1 / p62 antibody

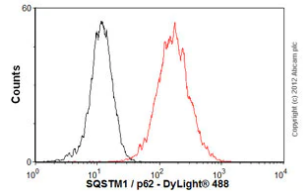
ab56416 (1µg/ml) staining SQSTM1 in human lymph node using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear and cytoplasmic staining.
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH 6.0 in a DAKO PT link. Slides were peroxidase 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.



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

SQSTM1 / p62 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-SQSTM1 / p62 antibody

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.



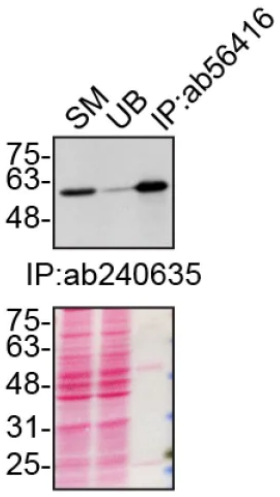
Flow Cytometry - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

SQSTM1 / p62 Flow Cytometry staining using mouse Anti-SQSTM1 / p62 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.

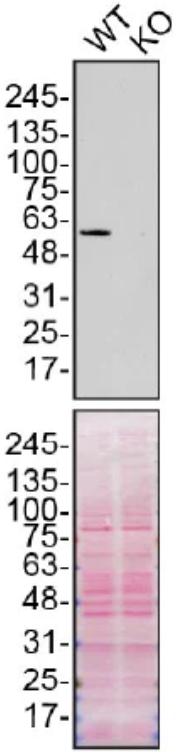
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Immunoprecipitation - Anti-SQSTM1 / p62 antibody [2C11] - BSA and Azide free (ab56416)

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

Predicted band size: 47 kDa



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

SQSTM1 / p62 Western blot staining using mouse Anti-SQSTM1 / p62 antibody

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

Lane 1:
Wild-type U-2 OS cell lysate at 20 µg

Lane 2:
SQSTM1 knockout U-2 OS cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 47 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.