

Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker ab109012

敲除验证
重组
RabMAb

★★★★★
[15 Abreviews](#)
[325 References](#)
[16 图像](#)

概述

产品名称	Anti-SQSTM1 / p62抗体[EPR4844] - Autophagosome Marker
描述	兔单克隆抗体[EPR4844] to SQSTM1 / p62 - Autophagosome Marker
宿主	Rabbit
特异性	This antibody got too weak band in rat tissues, you may need to optimize experimental protocols (increasing lysate amount, using lower dilution or higher sensitivity ECL substrate) to get visible band. However, it performs very well in rat cell lines.
经测试应用	适用于: WB, Flow Cyt (Intra), IP, ICC/IF
种属反应性	与反应: Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: U-2 OS, MCF-7, HAP1, HCT116, HeLa, HepG2, SKBR-3, MCF-7 and HEK-293T cell lysates; Mouse and rat brain, heart and lung tissue lysates. ICC/IF: HeLa cells (untreated and treated with chloroquine or bafilomycin) and HAP1 cells. Flow Cyt (intra): HeLa cells. IP: U-2 OS cell lysate.
常规说明	<p>We would like to recommend the following products as good alternatives to ab109012 that perform well in Immunocytochemistry/ Immunofluorescence:</p> <p>ab240635 and ab207305</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

性能

形式	Liquid
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存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR4844
同种型	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab109012于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (9)	1/10000 - 1/50000. Detects a band of approximately 62 kDa.
Flow Cyt (Intra)		1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP	★★★★★ (2)	Use at an assay dependent concentration.
ICC/IF		Use a concentration of 1 µg/ml.

靶标

功能	Adapter protein which binds ubiquitin and may regulate the activation of NFκB1 by TNF-α, 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.
组织特异性	Ubiquitously expressed.
疾病相关	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.
序列相似性	Contains 1 OPR domain. Contains 1 UBA domain. Contains 1 ZZ-type zinc finger.
结构域	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.

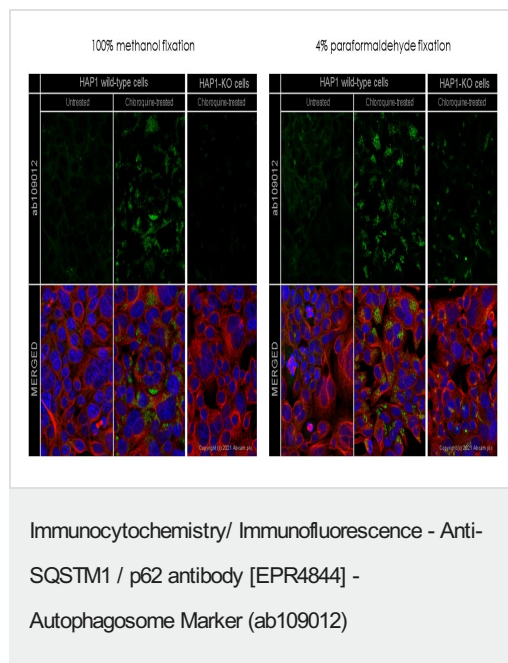
Phosphorylated. May be phosphorylated by PRKCZ (By similarity). Phosphorylated in vitro by TTN.

翻译后修饰

细胞定位

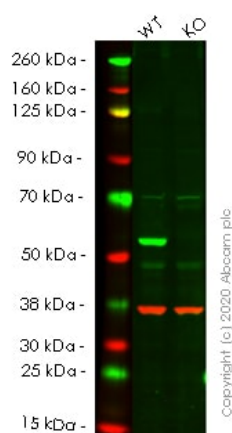
Cytoplasm. Late endosome. Nucleus. Sarcomere (By similarity). In cardiac muscles localizes to the sarcomeric band (By similarity). Localizes to late endosomes. May also localize to the nucleus. Accumulates in neurofibrillary tangles and in Lewy bodies of neurons from individuals with Alzheimer and Parkinson 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.

图片



ab109012 staining SQSTM1 in wild-type Hap1 cells and SQSTM1 knockout Hap1 cells treated with chloroquine ([ab142116](#), 50μM for 24 hrs). The cells were fixed with 100% methanol (5 min) or with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109012 at 0.1μg/ml concentration and [ab7291](#) (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) ([ab150081](#)) at 2 μg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) ([ab150120](#)) at 2 μg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

Residual signal is observed in KO cells when paraformaldehyde is used for fixation, therefore we recommend using methanol fixation with this antibody. Alternatively, please use [ab240635](#) or [ab207305](#) which have been KO validated in both paraformaldehyde and methanol fixed cells.



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

All lanes : Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/10000 dilution

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : SQSTM1 knockout HEK293T cell lysate

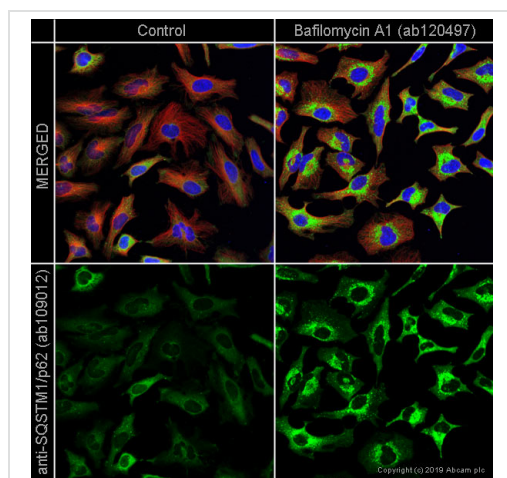
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 64 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109012 observed at 64 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

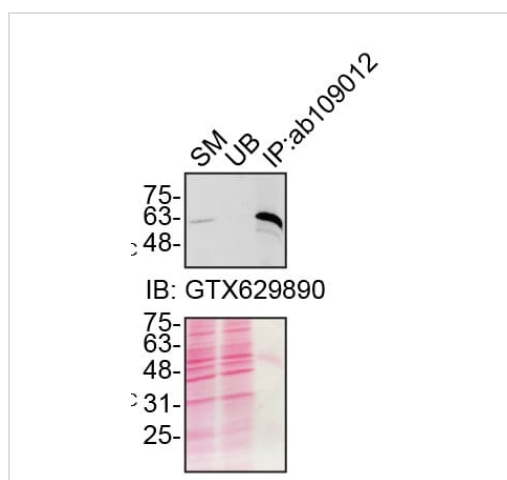
ab109012 was shown to react with SQSTM1/p62 in wild-type HEK293T cells in western blot. Loss of signal was observed when knockout cell line [ab255343](#) (knockout cell lysate [ab263770](#)) was used. Wild-type HEK293T and SQSTM1 knockout HEK293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109012 and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

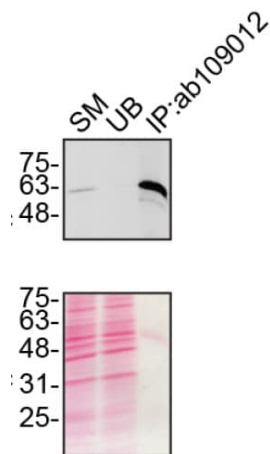
ab109012 staining SQSTM1/p62 (autophagosome) in control HeLa cells (left panel) and SQSTM1/p62 in HeLa cells treated with 1uM bafilomycin A1 (**ab120497**) for 18hrs (right panel). The cells were fixed with methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109012 at 5ug/ml and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



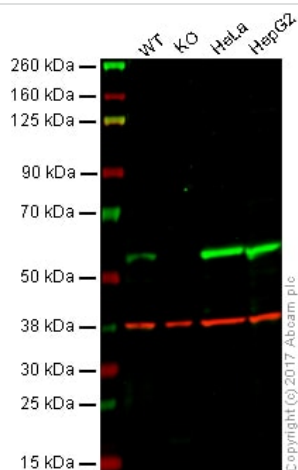
Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

Immunoprecipitation of SQSTM1 in U2OSn cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab109012 pre-coupled to prot.A-Sepharose beads. Samples were washed and processed for western blot with GTX629890 at 1/5000. 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 knock out cell lines.



Immunoprecipitation - Anti-SQSTM1 / p62 antibody
[EPR4844] - Autophagosome Marker (ab109012)

Immunoprecipitation of SQSTM1 in U-2 OS cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab109012 pre-coupled to prot.A-Sepharose beads. Samples were washed and processed for western blot with SQSTM1 / P62 antibody at 1/5000. 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
[EPR4844] - Autophagosome Marker (ab109012)

All lanes : Anti-SQSTM1 / p62 antibody [EPR4844] -
Autophagosome Marker (ab109012) at 1/10000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate

Lane 2 : SQSTM1 knockout HAP1 whole cell lysate

Lane 3 : HeLa whole cell lysate

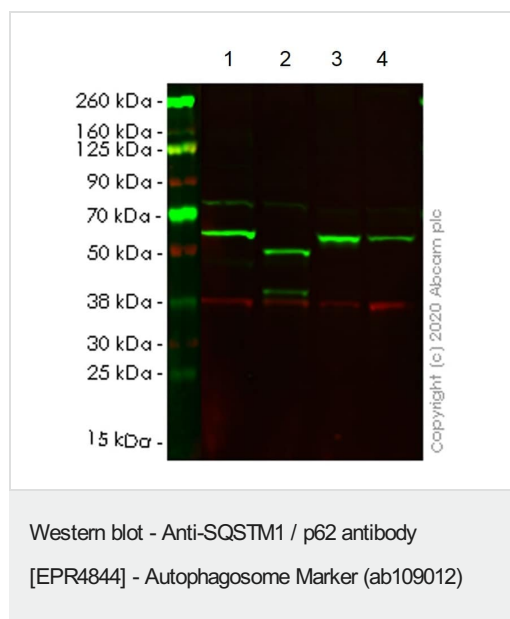
Lane 4 : HepG2 whole cell lysate

Lysates/proteins at 20 µg per lane.

Lanes 1 - 4: Merged signal (red and green). Green - ab109012 observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab109012 was shown to specifically react with SQSTM1 in wild-type HAP1 cells. No band was observed when SQSTM1 knockout samples were used. Wild-type and SQSTM1 knockout samples were subjected to SDS-PAGE, Ab109012 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/20,000 dilution respectively. Blots were

developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed [ab216773](#) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed [ab216776](#) secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.



All lanes : Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

Lane 1 : Wild-type HCT116 cell lysate

Lane 2 : SQSTM1 CRISPR/Cas9 edited HCT116 cell lysate

Lane 3 : HepG2 cell lysate

Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

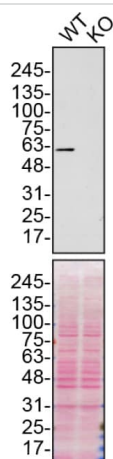
Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

Observed band size: 55 kDa

Lanes 1-4: Merged signal (red and green). Green - ab109012 observed at 55 kDa. Red - loading control [ab8245](#) observed at 36 kDa.

ab109012 Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker was shown to specifically react with SQSTM1 / p62 in wild-type HCT116 cells. The band observed in CRISPR/Cas9 edited cell line [ab266871](#) (CRISPR/Cas9 edited cell lysate [ab257052](#)) lane below 55 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and SQSTM1 / p62 CRISPR/Cas9 edited samples were subjected to SDS-PAGE. ab109012 and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-SQSTM1 / p62 antibody
[EPR4844] - Autophagosome Marker (ab109012)

All lanes : Anti-SQSTM1 / p62 antibody [EPR4844] -
Autophagosome Marker (ab109012) at 1/10000 dilution

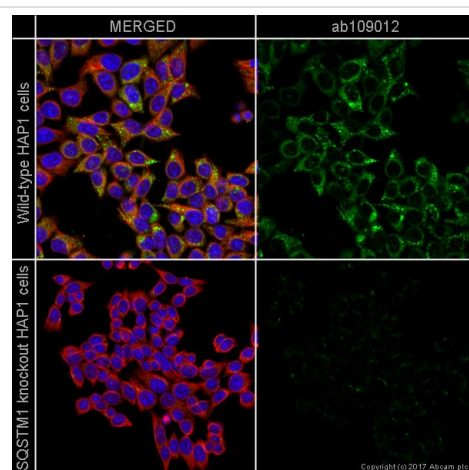
Lane 1 : Wild-type U-2 OS cell lysate

Lane 2 : SQSTM1 knockout U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

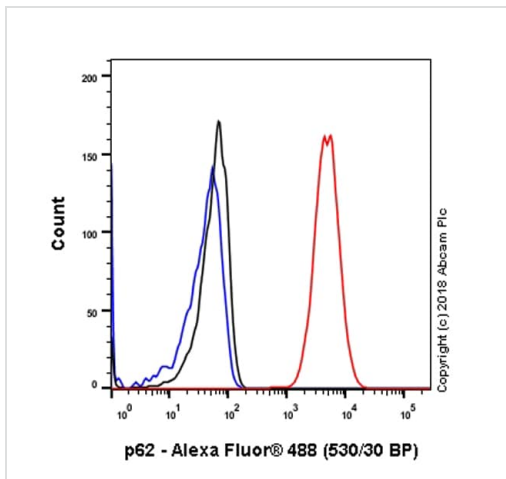
ab109012 was shown to react with SQSTM1 in wild-type U-2 OS cells in Western blot with loss of signal observed in a SQSTM1 knockout cell line. Wild-type U-2 OS and SQSTM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab109012 overnight at 4 °C at a 1/10000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 0.2µg/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 antibody [EPR4844] -
Autophagosome Marker (ab109012)

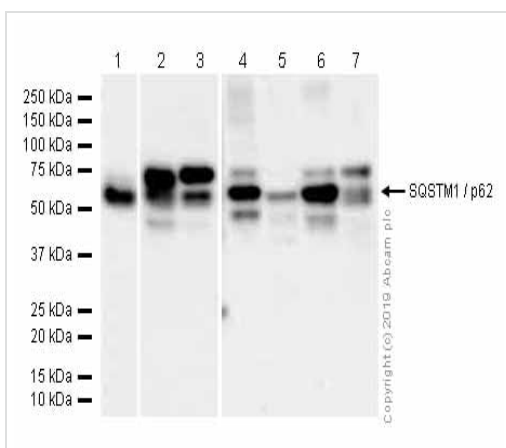
Purified ab109012 staining SQSTM1 in wild-type HAP1 cells (top panel) and SQSTM1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109012 at 1µg/ml and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling SQSTM1 / p62 with purified ab109012 at 1/50 dilution (10 µg/ml) (Red). Cells were fixed with 80% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012)

All lanes : Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (ab109012) at 1/1000 dilution (Purified)

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lane 2 : Mouse brain lysates

Lane 3 : rat brain lysates

Lane 4 : Mouse lung lysates

Lane 5 : Rat lung lysates

Lane 6 : Mouse heart lysates

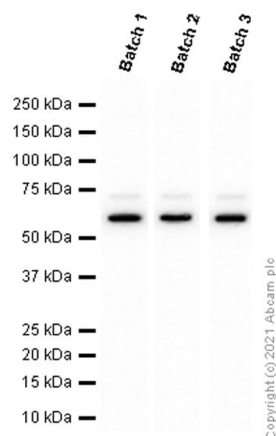
Lane 7 : Rat heart lysates

Lysates/proteins at 20 µg per lane.

Secondary

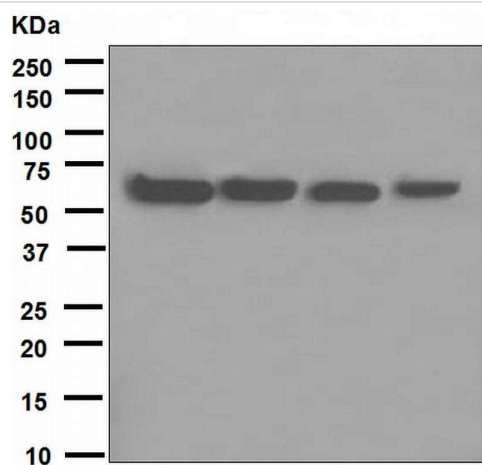
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Observed band size: 62 kDa



Western blot - Anti-SQSTM1 / p62 antibody
[EPR4844] - Autophagosome Marker (ab109012)

Different batches of ab109012 were tested on MCF7 (Human breast adenocarcinoma epithelial cell) lysate at 0.4 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 62 kDa.



Western blot - Anti-SQSTM1 / p62 antibody
[EPR4844] - Autophagosome Marker (ab109012)

All lanes : Anti-SQSTM1 / p62 antibody [EPR4844] -
Autophagosome Marker (ab109012) (Unpurified)

Lane 1 : MCF-7

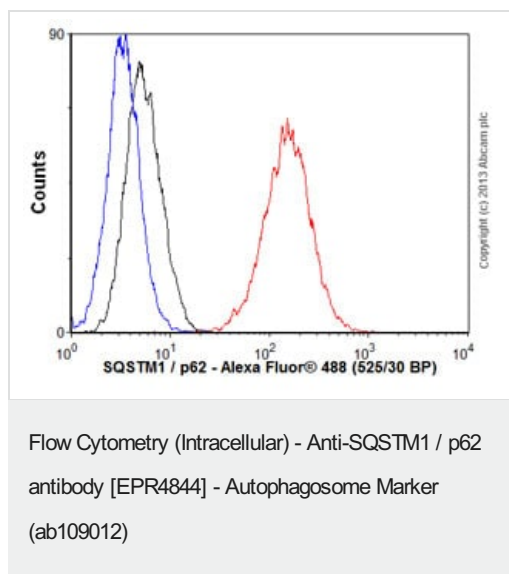
Lane 2 : HeLa

Lane 3 : SKBR-3

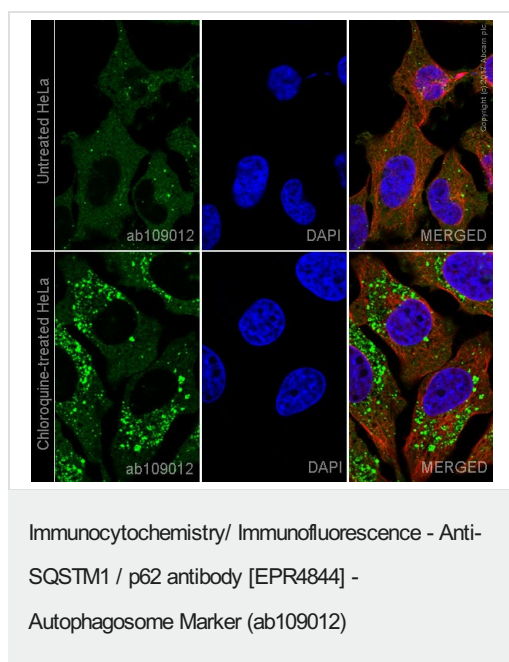
Lane 4 : 293T

Lysates/proteins at 10 µg per lane.

Observed band size: 62 kDa



Overlay histogram showing HeLa cells stained with unpurified ab109012 (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 (ab109012, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) ([ab150077](#)) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Unpurified ab109012 staining SQSTM1/p62 in HeLa cells +/- Chloroquine (50µM, 24 hours). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab109012 at 1µg/ml and [ab195889](#), Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in pseudocolor red) followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) ([ab150081](#)) at 2 µg/ml (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



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Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-SQSTM1 / p62 antibody [EPR4844] -
Autophagosome Marker (ab109012)

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