abcam

Product datasheet

Anti-SQSTM1 / p62 antibody [EPR18351] ab207305

KO 評価済 リコンビナント RabMAb

3 Abreviews 39 References 画像数 16

製品の概要

製品名	Anti-SQSTM1 / p62 antibody [EPR18351]
製品の詳細	Rabbit monoclonal [EPR18351] to SQSTM1 / p62
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), IHC-P, WB, ICC/IF, IP
種交差性	**交差種:** Human
免疫原	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Human liver and kidney lysates; U-2 OS, HCT116, HepG2 and HeLa whole cell lysates. IHC-P: Human hepatocellular carcinoma and lung cancer tissues. ICC/IF: HeLa cells (untreated and treated with chloroquine), HAP1 cells (untreated and treated with chloroquine), U-2 OS cells. Flow Cyt (intra): PC-3 cells. IP: HeLa and HepG2 whole cell lysates.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR18351

アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab207305の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 62 kDa (predicted molecular weight: 47 kDa).
ICC/IF	★★★★★ (1)	Use a concentration of 1 µg/ml.
IP	★★★★★ (2)	1/40.

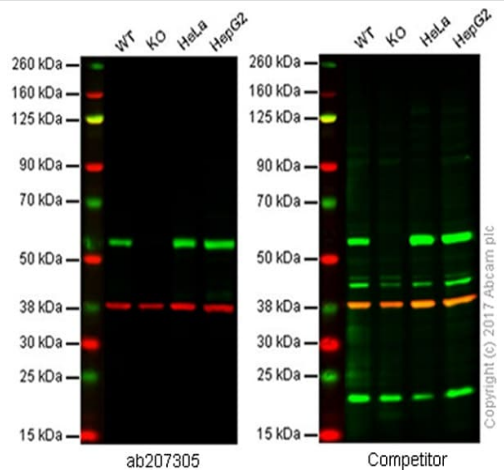
ターゲット情報

機能	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.
組織特異性	Ubiquitously expressed.
関連疾患	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.
配列類似性	Contains 1 OPR domain. Contains 1 UBA domain. Contains 1 ZZ-type zinc finger.
ドメイン	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.
翻訳後修飾	Phosphorylated. May be phosphorylated by PRKCZ (By similarity). Phosphorylated in vitro by TTN.

細胞内局在

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.

画像

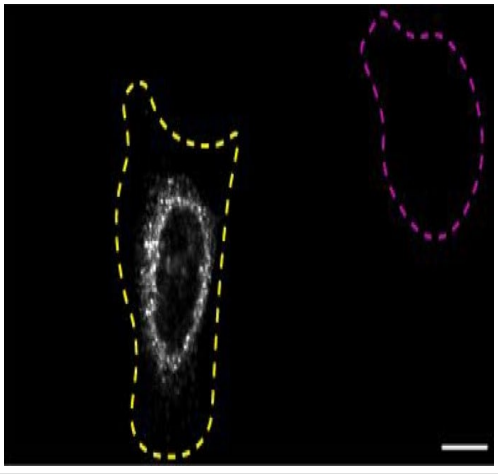


Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: SQSTM1/p62 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green).

Green - target observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

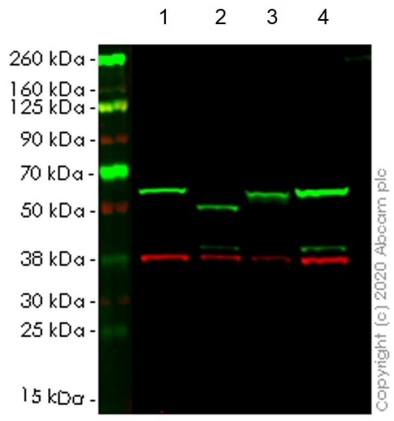
This western blot image is a comparison between ab207305 and a competitor's discontinued goat polyclonal antibody.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

ab207305 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 1/5000. The cells were then incubated with ab207305 at 1/200 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit 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.



Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

All lanes : Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

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

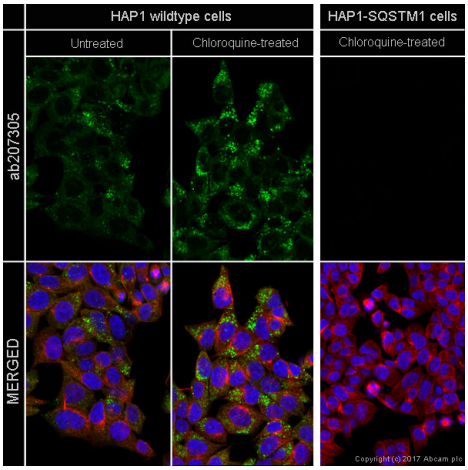
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 47 kDa
Observed band size: 55 kDa

Lanes 1-4: Merged signal (red and green). Green - ab207305 observed at 55 kDa. Red - loading control **ab8245** observed at 36 kDa.

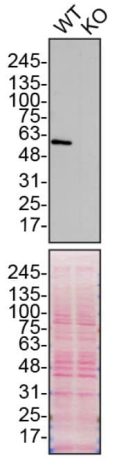
ab207305 Anti-SQSTM1 / p62 antibody [EPR18351] was shown to specifically react with SQSTM1 / p62 in wild-type HCT116 cells. The band observed in CRISPR/Cas9 edited cell line **ab266871** (CRISPR/Cas9 edited cell lysate **ab257052**) lane below 55 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and SQSTM1 / p62 CRISPR/Cas9 edited samples were subjected to SDS-PAGE. ab207305 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.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

ab207305 staining SQSTM1 in wild-type HAP1 cells and knockout cells, untreated and chloroquine-treated (**ab142116**, 50µM, 24 hours). The cells were fixed with 4% formaldehyde (10min), 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 ab207305 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).



Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

All lanes : Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305) at 1/1000 dilution

Lane 1 : Wild-type U-2 OS cell lysate
Lane 2 : SQSTM1 knockout U-2 OS cell lysate

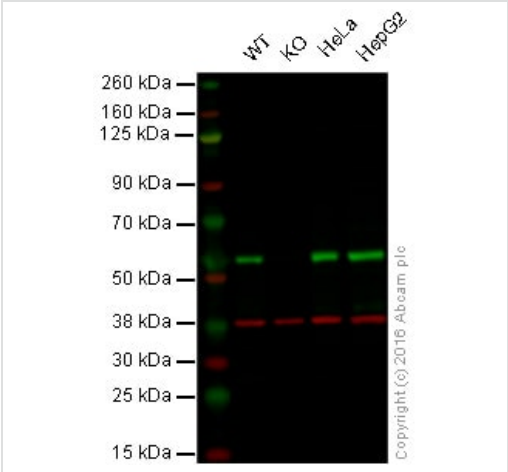
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa

ab207305 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 ab207305 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-rabbit 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

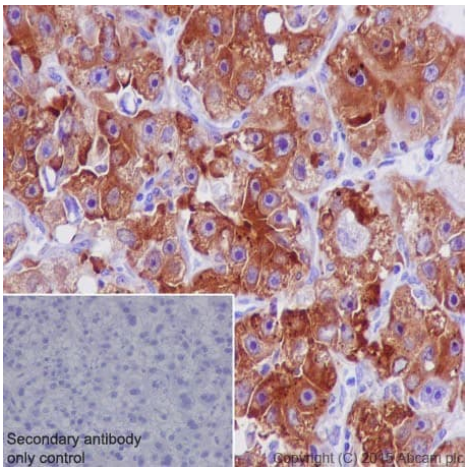
working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: SQSTM1/p62 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab207305 observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab207305 was shown to specifically react with SQSTM1/p62 in wild-type HAP1 cells. No band was observed when SQSTM1/p62 knockout samples were used. Wild-type and SQSTM1/p62 knockout samples were subjected to SDS-PAGE, ab207305 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10,000 respectively and incubated overnight at 4°C. 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 1/10,000 dilution for 1 hour at room temperature before imaging.

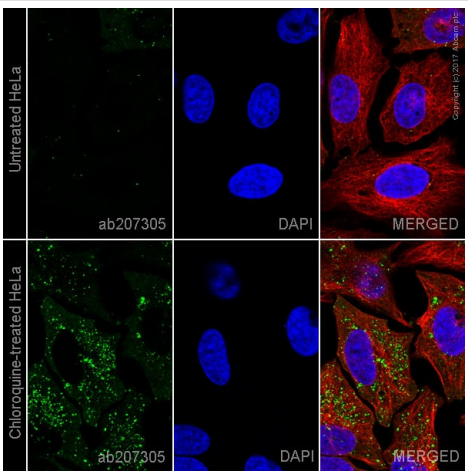


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma tissue labeling SQSTM1 / p62 with ab207305 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasm staining on human hepatocellular carcinoma is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

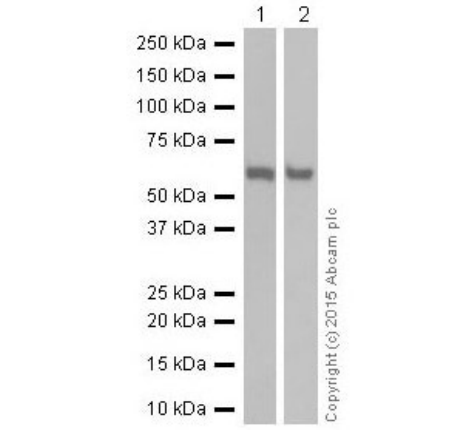
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

ab207305 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 ab207305 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).



Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

All lanes : Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305) at 1/1000 dilution

Lane 1 : Human liver lysate
Lane 2 : Human kidney lysate

Lysates/proteins at 10 µg per lane.

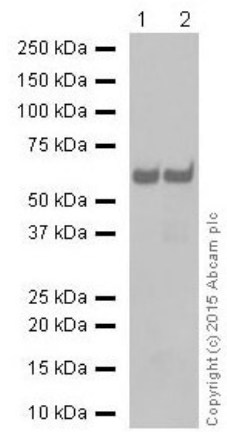
Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 47 kDa
Observed band size: 62 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The MW observed is consistent with what has been described in the literature (PMID: PMC4344198).



Western blot - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

All lanes : Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305) at 1/5000 dilution

Lane 1 : HepG2 (Human liver hepatocellular carcinoma) whole cell lysate
Lane 2 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 10 µg per lane.

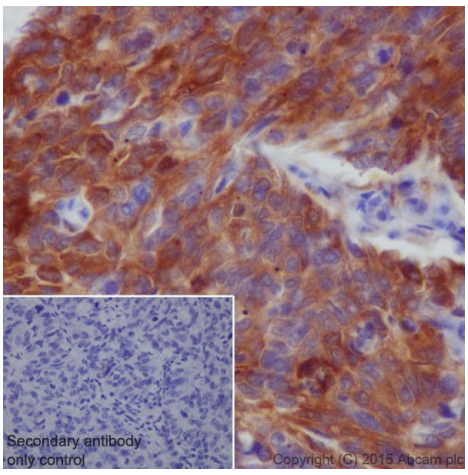
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 47 kDa
Observed band size: 62 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The MW observed is consistent with what has been described in the literature (PMID: PMC4344198).

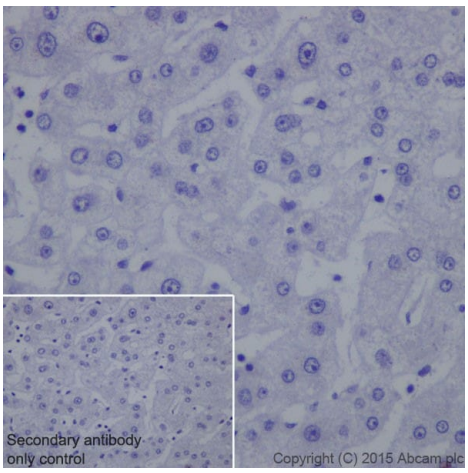


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling SQSTM1 / p62 with ab207305 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasm and weakly nucleus staining on human lung cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

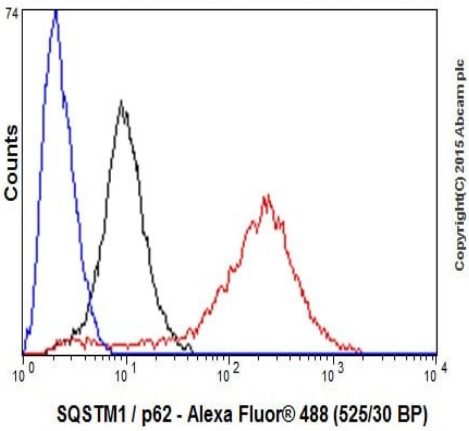


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling SQSTM1 / p62 with ab207305 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Negative staining on Hhman normal liver tissue. Counter stained with Hematoxylin.

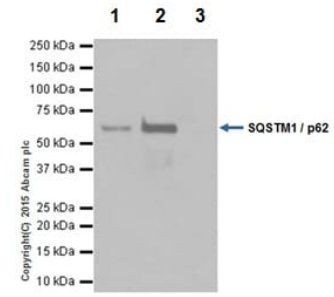
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



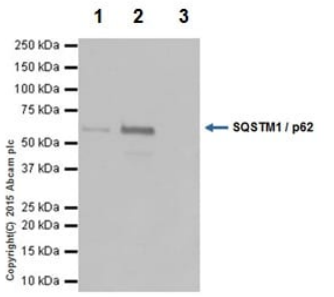
Flow Cytometry (Intracellular) - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed PC-3 (Human prostate adenocarcinoma cell line) cells labeling SQSTM1 / p62 with ab207305 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat Anti-Rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

SQSTM1 / p62 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab207305 at 1/40 dilution.
Western blot was performed from the immunoprecipitate using ab207305 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.
Lane 1: HeLa whole cell lysate 10ug (Input).
Lane 2: ab207305 IP in HeLa whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab207305 in HeLa whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 1 second.



Immunoprecipitation - Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

SQSTM1 / p62 was immunoprecipitated from 1mg of HepG2 (Human liver hepatocellular carcinoma) whole cell lysate with ab207305 at 1/40 dilution.
Western blot was performed from the immunoprecipitate using ab207305 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.
Lane 1: HepG2 whole cell lysate 10ug (Input).
Lane 2: ab207305 IP in HepG2 whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab207305 in HepG2 whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 1 second.

Anti-SQSTM1 / p62 antibody [EPR18351] (ab207305)

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