abcam

Product datasheet

Human SQSTM1 (p62) knockout HCT116 cell line ab266871

4 Images

Overview

Product name	Human SQSTM1 (p62) knockout HCT116 cell line
Parental Cell Line	HCT116
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 49 bp deletion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **Recommended control:** Human wild-type HCT116 cell line (**ab255451**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** McCoY5a + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:**

All seeding densities should be based on cell counts gained by established methods.
A guide seeding density of $2x10^4$ cells/cm^2 is recommended.
A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Colon
Cell type	epithelial
Disease	Carcinoma
Gender	Male
STR Analysis	Amelogenin X D5S818: 10, 11 D13S317: 10, 12 D7S820: 11, 12 D16S539: 11, 13 vWA: 17, 22 TH01: 8,9 TPOX: 8, 9 CSF1PO: 7, 10
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.
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.

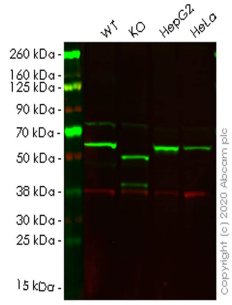
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266871 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 47 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human SQSTM1 (p62) knockout HCT116 cell line (ab266871)

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

Lane 1 : Wild-type HCT116 cell lysate

Lane 2 : SQSTM1 knockout HCT116 cell lysate

Lane 3 : HepG2 cell lysate

Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

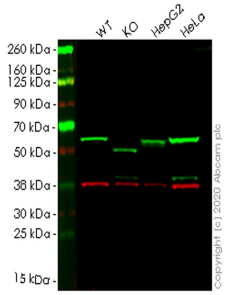
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 47 kDa

Observed band size: 55 kDa

Lanes 1-4: Merged signal (red and green). Green - **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 knockout cell line ab266871 (knockout 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 knockout 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 - Human SQSTM1 (p62) knockout HCT116 cell line (ab266871)

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

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : SQSTM1 knockout HCT116 cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

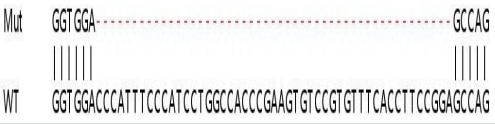
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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.

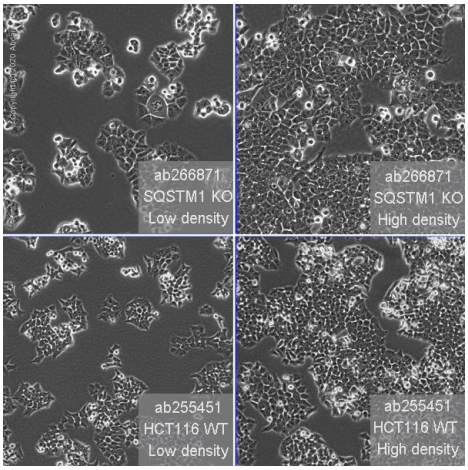
ab207305 Anti-SQSTM1 / p62 antibody [EPR18351] was shown to specifically react with SQSTM1 / p62 in wild-type HCT116 cells. The band observed in knockout cell line ab266871 (knockout cell

lysate **ab257052**) lane below 55 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and SQSTM1 / p62 knockout 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.



Sanger Sequencing - Human SQSTM1 knockout HCT116 cell line (ab266871)

Homozygous: 49 bp deletion in exon4



Cell Culture - Human SQSTM1 (p62) knockout HCT116 cell line (ab266871)

Representative images of SQSTM1 knockout HCT116 cells, low and high confluency examples (top left and right respectively) and wild-type HCT116 cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.

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