

Product datasheet

Human SQSTM1 (p62) knockout HEK-293T cell lysate ab263770

3 Images

Overview

Product name	Human SQSTM1 (p62) knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 4.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

[See here for more information on knockout cell lysates.](#)

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Tested applications **Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab255452 - Human SQSTM1 knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12

Target

Function Adapter protein which binds ubiquitin and may regulate the activation of NFκB1 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

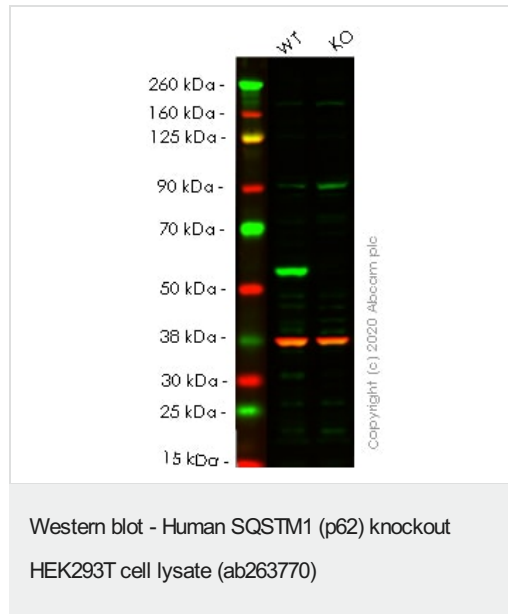
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab263770 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.

Images

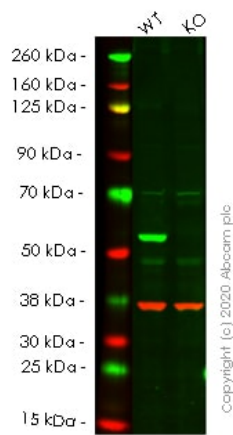


Lane 1: Wild-type HEK293T cell lysate (20µg)

Lane 2: SQSTM1 knockout HEK293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab56416** observed at 62 kDa. Red - loading control **ab181602** observed at 37 kDa.

ab56416 Anti-SQSTM1 / p62 antibody - Autophagosome Marker was shown to specifically react with SQSTM1 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 and SQSTM1 knockout samples 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**) were incubated overnight at 4 °C at 1 in 1000 dilution and 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.



Western blot - Human SQSTM1 (p62) knockout
HEK293T cell lysate (ab263770)

Lane 1: Wild-type HEK293T cell lysate (20µg)

Lane 2: SQSTM1 knockout HEK293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab109012** observed at 64 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab109012 Recombinant Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker was shown to specifically react with SQSTM1 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 and SQSTM1 knockout samples 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**) were incubated overnight at 4 °C at 1 in 10000 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.

Homozygous: 1 bp insertion in exon 4

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Mut  GCCCACCCTGTGCTCAGGAGGCGCCCCGAAACATGGTGCACCCCAATGTGATCTGCGAT
      |||
WT   GCCCACCCTGTGCTCAGGAGGCGCCCCGCAA CATGGTGCACCCCAATGTGATCTGCGAT
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Sanger Sequencing - Human SQSTM1 knockout
HEK293T cell lysate (ab263770)

Homozygous: 1 bp insertion in exon 4

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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