abcam

Product datasheet

Human SQSTM1 (p62) knockout HEK-293T cell line ab255343

4 图像

概述

产品名称	人SQSTM1 (p62) knockout HEK-293T cell line
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)
经测试应用	**适用于:** WB
Biosafety level	2
常规说明	**Recommended control:** Human wild-type HEK293T cell line (**ab255449**). 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:** DMEM (High Glucose) + 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, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

性能

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
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
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	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.
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.
翻译后修饰	Phosphorylated. May be phosphorylated by PRKCZ (By similarity). Phosphorylated in vitro by TTN.
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.

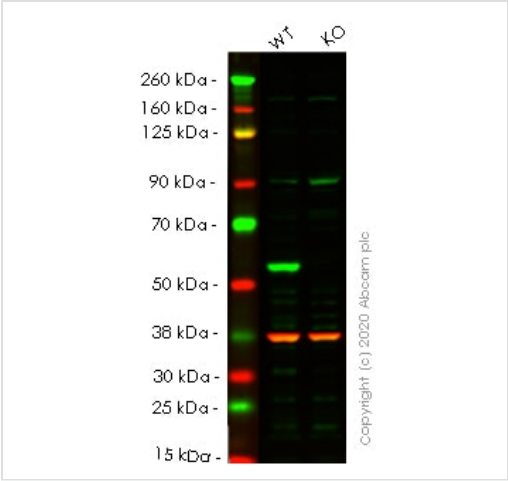
应用

The Abpromise guarantee **Abpromise™承诺保证使用ab255343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		Use at an assay dependent concentration. Predicted molecular weight: 47 kDa.

图片



Western blot - Human SQSTM1 (p62) knockout HEK293T cell line (ab255343)

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

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : SQSTM1 knockout HEK293T cell lysate

Lysates/proteins at 20 µg per lane.

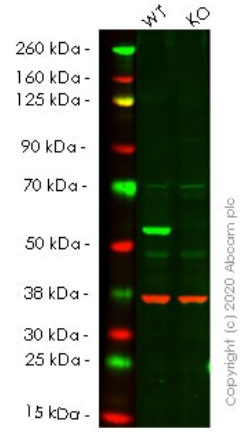
Performed under reducing conditions.

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

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 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.



Western blot - Human SQSTM1 (p62) knockout HEK293T cell line (ab255343)

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.

Predicted band size: 47 kDa
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 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.

Sanger Sequencing - Human SQSTM1 (p62) knockout HEK-293T cell line (ab255343)

Sequencing chromatogram displaying sequence edit in exon 4

Sanger Sequencing - Human SQSTM1 knockout HEK293T cell line (ab255343)

Homozygous: 1 bp insertion in exon 4

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors