abcam

Product datasheet

Human ATG16L1 knockout HeLa cell line ab261773

3 Images

Overview

Product name	Human ATG16L1 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 22 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). 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 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	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 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	Plays an essential role in autophagy: interacts with ATG12-ATG5 to mediate the conjugation of phosphatidylethanolamine (PE) to LC3 (MAP1LC3A, MAP1LC3B or MAP1LC3C), to produce a membrane-bound activated form of LC3 named LC3-II. Thereby, controls the elongation of the nascent autophagosomal membrane.
Involvement in disease	Inflammatory bowel disease 10
Sequence similarities	Belongs to the WD repeat ATG16 family. Contains 7 WD repeats.
Post-translational modifications	Proteolytic cleavage by activated CASP3 leads to degradation and may regulate autophagy upon cellular stress and apoptotic stimuli.
Cellular localization	Cytoplasm. Preautophagosomal structure membrane. Recruited to omegasomes membranes by WIPI2. Omegasomes are endoplasmic reticulum connected strutures at the origin of preautophagosomal structures. Localized to preautophagosomal structure (PAS) where it is involved in the membrane targeting of ATG5. Localizes also to discrete punctae along the ciliary axoneme.
Form	There are 4 isoforms produced by alternative splicing.

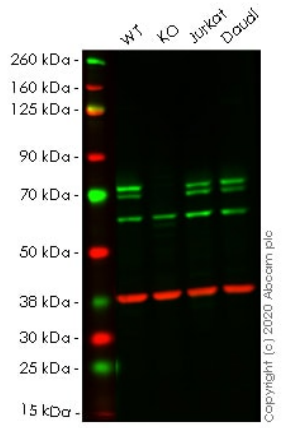
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261773 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: 68 kDa.

Images



Western blot - Human ATG16L1 knockout HeLa cell line (ab261773)

All lanes : Anti-ATG16L1 antibody [5H9A11] (**ab233796**) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG16L1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

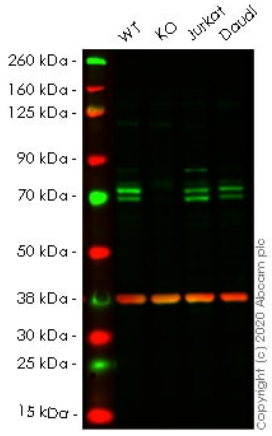
Secondary
All lanes : Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 68 and 72 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab233796** observed at 68 and 72 kDa. Red - loading control **ab181602** observed at 37 kDa.

ab233796 Anti-ATG16L1 antibody [5H9A11] was shown to specifically react with ATG16L1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261773 (knockout cell lysate **ab256844**) was used. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. **ab233796** and Anti-GAPDH antibody[EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 500 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 ATG16L1 knockout HeLa cell line (ab261773)

All lanes : Anti-ATG16L1 antibody [EPR15638] - N-terminal (**ab187671**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG16L1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 68 and 72 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab187671** observed at 68 and 72 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab187671 Anti-ATG16L1 antibody [EPR15638] - N-terminal was shown to specifically react with ATG16L1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261773 (knockout cell lysate **ab256844**) was used. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. **ab187671** 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 ATG16L1 knockout HeLa cell line (ab261773)

Homozygous: 22 bp deletion in exon 1.

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

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