abcam

Product datasheet

Human ATG4B knockout HeLa cell line ab265814

4 Images

Overview

Product name	Human ATG4B knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 4 and 1 bp insertion in exon 4
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.
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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
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	Cysteine protease required for autophagy, which cleaves the C-terminal part of either MAP1LC3, GABARAPL2 or GABARAP, allowing the liberation of form I. A subpopulation of form I is subsequently converted to a smaller form (form II). Form II, with a revealed C-terminal glycine, is considered to be the phosphatidylethanolamine (PE)-conjugated form, and has the capacity for the binding to autophagosomes.
Tissue specificity	Mainly expressed in the skeletal muscle, followed by brain, heart, liver and pancreas.
Sequence similarities	Belongs to the peptidase C54 family.
Cellular localization	Cytoplasm.

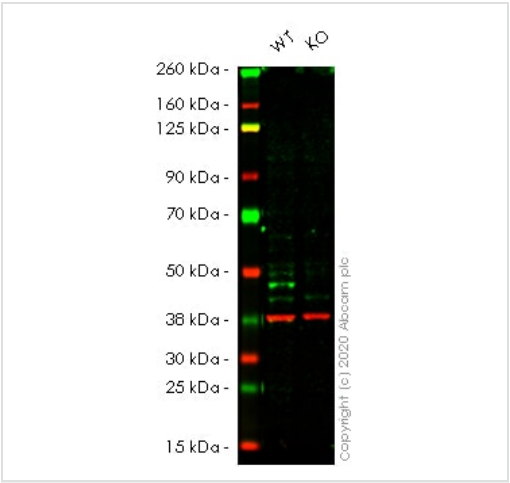
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265814 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: 44 kDa.

Images



Western blot - Human ATG4B knockout HeLa cell line (ab265814)

All lanes : Anti-ATG4B antibody [EPR16572] (**ab199537**) at 1/1000 dilution

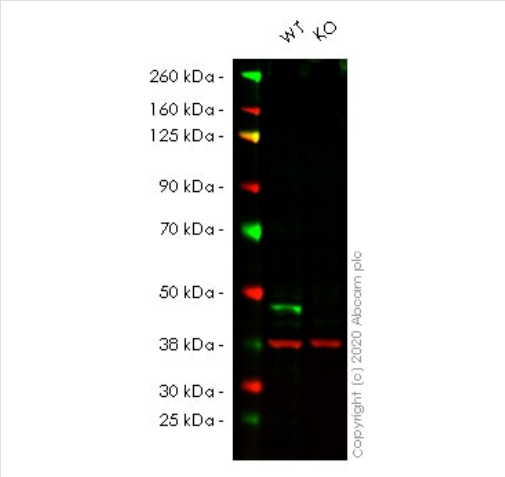
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG4B knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 44 kDa
Observed band size: 47 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab199537** observed at 47 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab199537 was shown to react with ATG4B in wild-type HeLa cells in Western blot with loss of signal observed in ATG4B knockout cell line **ab260973** (ATG4B knockout cell lysate **ab257364**). Wild-type and ATG4B knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab199537** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human ATG4B knockout HeLa cell line (ab265814)

All lanes : Anti-ATG4B antibody [EPR6436(2)] (**ab154843**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG4B knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 44 kDa
Observed band size: 47 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab154843** observed at 47 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab154843 was shown to react with ATG4B in wild-type HeLa cells in Western blot with loss of signal observed in ATG4B knockout cell line **ab260973** (ATG4B knockout cell lysate **ab257364**). Wild-type and ATG4B knockout HEK293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab154843** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Sanger Sequencing - Human ATG4B knockout HeLa cell line (ab265814)

Allele-1: 1 bp deletion in exon 4.

Sanger Sequencing - Human ATG4B knockout HeLa cell line (ab265814)

Allele-2: 1 bp insertion in exon 4.

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