# abcam

### Product datasheet

# Human ATG16L1 knockout HeLa cell line ab261773

#### 3 图像

#### 概述

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)

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经测试应用 适用于: WB

Biosafety level

常规说明

**Recommended control:** Human wild-type HeLa cell line (<u>ab255928</u>). 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<sup>4</sup> cells/cm<sup>2</sup>. 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<sub>2</sub>. 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<sup>2</sup> is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

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Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

#### 性能

Number of cells 1 x 10<sup>6</sup> 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

**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 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.

疾病相关 Inflammatory bowel disease 10

序列相似性 Belongs to the WD repeat ATG16 family.

Contains 7 WD repeats.

翻译后修饰 Proteolytic cleavage by activated CASP3 leads to degradation and may regulate autophagy upon

cellular stress and apoptotic stimuli.

细胞定位 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.

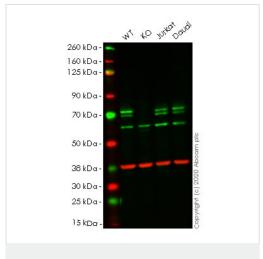
形式 There are 4 isoforms produced by alternative splicing.

## 应用

The Abpromise guarantee Abpromise™承诺保证使用ab261773于以下的经测试应用

应 <b>用</b>	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 68 kDa.

#### 图片



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

**All lanes :** Anti-ATG16L1 antibody [5H9A11] (<u>ab233796</u>) 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

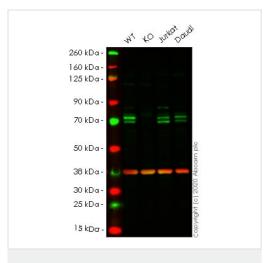
Performed under reducing conditions.

Predicted band size: 68 kDa

Observed band size: 68 and 72 kDa

**Lanes 1-4:** Merged signal (red and green). Green - <u>ab233796</u> observed at 68 and 72 kDa. Red - loading control <u>ab181602</u> 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 lgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat Anti-Rabbit lgG 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 lgG 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 - <u>ab187671</u> observed at 68 and 72 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

<u>ab187671</u> 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 <u>ab256844</u>) was used. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. <u>ab187671</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Mut	CT GAGGT GCCGGGGCAGCAAGT GACAT GT CG	TCCCCCG		
WT	CTGAGGTGCCGGGGCAGCAAGTGACATGTCGTCGGGCCTCCGCGCCGCTGACT	TCCCCCG		
Sanger Sequencing - Human ATG16L1 knockout				
Canger Sequencing - Human ATO TOET Knockout				
HeLa cell line (ab261773)				

Homozygous: 22 bp deletion in exon 1.

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