

Human ATG16L1 knockout HeLa cell line ab265263

5 图像

概述

产品名称	人ATG16L1 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 1.
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	<p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

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 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 WWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
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 structures 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

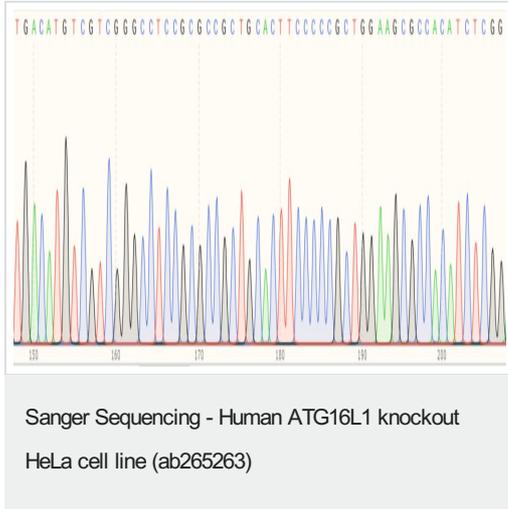
The Abpromise guarantee

Abpromise™ 承诺保证使用ab265263于以下的经测试应用

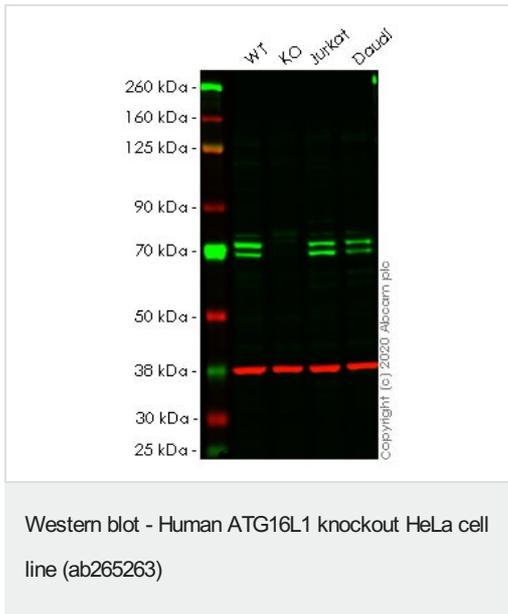
“应用说明”部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 68 kDa.

图片



Sequencing chromatogram displaying sequence edit in exon 1



All lanes : Anti-ATG16L1 antibody [5H9A11] ([ab233796](#)) 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.

Performed under reducing conditions.

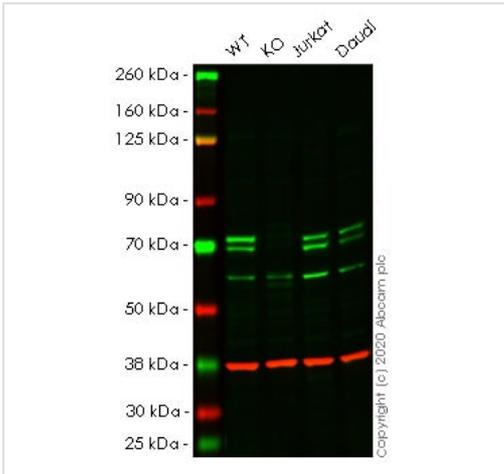
Predicted band size: 68 kDa

Observed band size: 68 kDa

Lanes 1- 4: Merged signal (red and green). Green - [ab233796](#) observed at 68 kDa. Red - Anti-GAPDH antibody[EPR16891] - Loading Control ([ab181602](#)) observed at 37 kDa.

[ab233796](#) was shown to react with ATG16L1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265263 (knockout cell lysate [ab256842](#)) was used. Wild-

type HeLa and ATG16L1 knockout HeLa 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. **ab233796** 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 ATG16L1 knockout HeLa cell line (ab265263)

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.

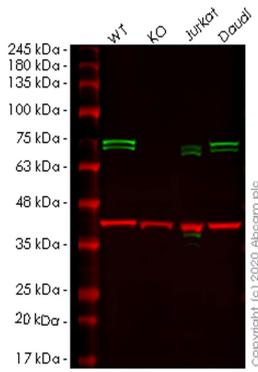
Performed under reducing conditions.

Predicted band size: 68 kDa

Observed band size: 68 kDa

Lanes 1- 4: Merged signal (red and green). Green - **ab187671** observed at 68 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab187671 was shown to react with ATG16L1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265263 (knockout cell lysate **ab256842**) was used. Wild-type HeLa and ATG16L1 knockout HeLa 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. **ab187671** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 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.



Western blot - Human ATG16L1 knockout HeLa cell line (ab265263)

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 1/10000 dilution

Predicted band size: 68 kDa

Observed band size: 75 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab187671** observed at 75 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab187671 Anti-ATG16L1 antibody [EPR15638] was shown to specifically react with ATG16L1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265263 (knockout cell lysate **ab256842**) was used. Wild-type and ATG16L1 knockout samples were subjected to SDS-PAGE. **ab187671** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours 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.

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Mut  TGACATGTCGTCGGGCCCTCCGGCCGCTGCACCTTCCCCCGCTGGAAGGCCACATCTCGG
      |||
WT   TGACATGTCGTCGGGCCCTCCGGCCGCTG ACTTCCCCCGCTGGAAGGCCACATCTCGG
  
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Sanger Sequencing - Human ATG16L1 knockout HeLa cell line (ab265263)

Allele-1: 1 bp insertion in exon 1.

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