abcam

Product datasheet

Human ATG7 knockout HeLa cell line ab283307

3 图像

概述

产品名称	人ATG7 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, functional Homozygous: 41 dp deletion 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 HeLa cell line (**ab275466**). 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.

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
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	E1-like activating enzyme involved in the 2 ubiquitin-like systems required for cytoplasm to vacuole transport (Cvt) and autophagy. Activates ATG12 for its conjugation with ATG5 as well as the ATG8 family proteins for their conjugation with phosphatidylethanolamine. Both systems are needed for the ATG8 association to Cvt vesicles and autophagosomes membranes. Required for autophagic death induced by caspase-8 inhibition. Required for mitophagy which contributes to regulate mitochondrial quantity and quality by eliminating the mitochondria to a basal level to fulfill cellular energy requirements and preventing excess ROS production. Modulates p53/TP53 activity to regulate cell cycle and survival during metabolic stress. Plays also a key role in the maintenance of axonal homeostasis, the prevention of axonal degeneration, the maintenance of hematopoietic stem cells, the formation of Paneth cell granules, as well as in adipose differentiation.
组织特异性	Widely expressed, especially in kidney, liver, lymph nodes and bone marrow.
序列相似性	Belongs to the ATG7 family.
结构域	The C-terminal part of the protein is essential for the dimerization and interaction with ATG3 and ATG12. The N-terminal FAP motif (residues 15 to 17) is essential for the formation of the ATG89-PE and ATG5-ATG12 conjugates.
翻译后修饰	Acetylated by EP300.
细胞定位	Cytoplasm. Preautophagosomal structure. Localizes also to discrete punctae along the ciliary axoneme and to the base of the ciliary axoneme.

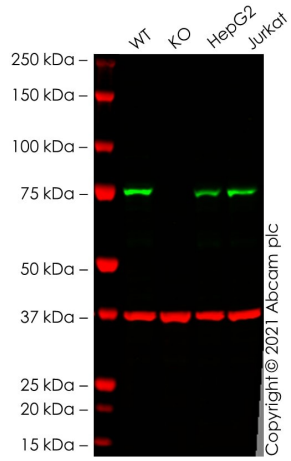
应用

The Abpromise guarantee **Abpromise™承诺保证使用ab283307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.

图片



Western blot - Human ATG7 knockout HeLa cell line (ab283307)

All lanes : Anti-ATG7 antibody [EPR6251] (**ab133528**) at 1/10000 dilution

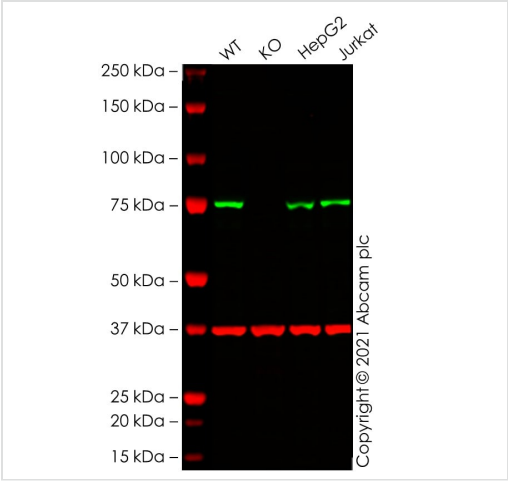
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG7 knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 75 kDa

False colour image of Western blot: Anti-ATG7 antibody [EPR6251] staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab133528** was shown to bind specifically to ATG7. A band was observed at 75 kDa in wild-type HeLa cell lysates with no signal observed at this size in ATG7 knockout cell line ab283307 (knockout cell lysate **ab287353**). To generate this image, wild-type and ATG7 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Human ATG7 knockout HeLa cell line (ab283307)

All lanes : Anti-ATG7 antibody [EP1759Y] (**ab52472**) at 1/100000 dilution

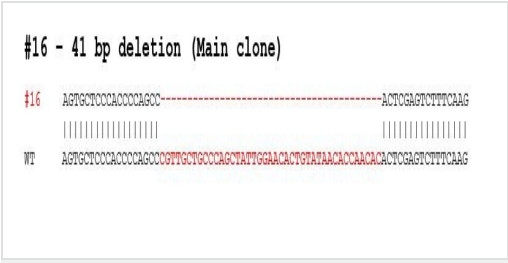
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ATG7 knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 75 kDa

False colour image of Western blot: Anti-ATG7 antibody [EP1759Y] staining at 1/100000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab52472** was shown to bind specifically to ATG7. A band was observed at 75 kDa in wild-type HeLa cell lysates with no signal observed at this size in ATG7 knockout cell line ab283307 (knockout cell lysate **ab287353**). To generate this image, wild-type and ATG7 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Sanger Sequencing - Human ATG7 knockout HeLa cell line (ab283307)

41 bp deletion in exon 4

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