abcam

Product datasheet

Human ATG3 knockout HEK-293T cell line ab266707

3 Images

Overview

Product name	Human ATG3 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 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 HEK293T cell line (**ab255449**). 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	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
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	E2-like enzyme involved in autophagy and mitochondrial homeostasis. Catalyzes the conjugation of ATG8-like proteins (GABARAP, GABARAPL1, GABARAPL2 or MAP1LC3A) to phosphatidylethanolamine (PE). PE-conjugation to ATG8-like proteins is essential for autophagy. Preferred substrate is MAP1LC3A. Also acts as an autocatalytic E2-like enzyme, catalyzing the conjugation of ATG12 to itself, ATG12 conjugation to ATG3 playing a role in mitochondrial homeostasis but not in autophagy. ATG7 (E1-like enzyme) facilitates this reaction by forming an E1-E2 complex with ATG3.
Tissue specificity	Widely expressed, with a highest expression in heart, skeletal muscle, kidney, liver and placenta.
Sequence similarities	Belongs to the ATG3 family.
Post-translational modifications	Conjugated to ATG12 at Lys-243. ATG12-conjugation plays a role in regulation of mitochondrial homeostasis and cell death, while it is not involved in PE-conjugation to ATG8-like proteins and autophagy.
Cellular localization	Cytoplasm.

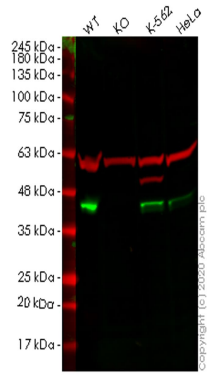
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266707 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: 35 kDa.

Images



Western blot - Human ATG3 knockout HEK293T cell line (ab266707)

All lanes : Anti-ATG3 antibody [EPR4801] (**ab108251**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : ATG3 knockout HEK293T cell lysate
Lane 3 : K-562 cell lysate
Lane 4 : HeLa cell lysate

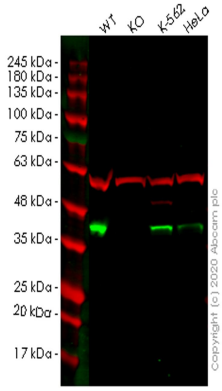
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 35 kDa
Observed band size: 40 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab108251** observed at 40 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab108251 Anti-ATG3 antibody [EPR4801] was shown to specifically react with ATG3 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266707 (knockout cell lysate **ab257363**) was used. Wild-type and ATG3 knockout samples were subjected to SDS-PAGE. **ab108251** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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.



Western blot - Human ATG3 knockout HEK293T cell line (ab266707)

All lanes : Anti-ATG3 antibody [EPR4802] (**ab108282**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : ATG3 knockout HEK293T cell lysate
Lane 3 : K-562 cell lysate
Lane 4 : HeLa 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: 35 kDa
Observed band size: 40 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab108282** observed at 40 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab108282 Anti-ATG3 antibody [EPR4802] was shown to specifically react with ATG3 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266707 (knockout cell lysate **ab257363**) was used. Wild-type and ATG3 knockout samples were subjected to SDS-PAGE. **ab108282** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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 ATG3 knockout HEK293T cell line (ab266707)

Homozygous: 1 bp deletion in exon 1

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

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