abcam

Product datasheet

# Human ATG4C knockout HEK-293T cell line ab267324

3 Images

## Overview

| | |
|---|---|
| **Product name** | Human ATG4C knockout HEK-293T cell line |
| **Parental Cell Line** | HEK293T |
| **Organism** | Human |
| **Mutation description** | Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 5 |
| **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.<br><br>**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.<br><br>**Culture medium:** DMEM (High Glucose) + 10% FBS<br><br>**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.<br><br>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.<br>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.<br>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.<br>4. Incubate the culture at 37°C incubator with 5% $CO_2$. Cultures should be monitored daily.<br><br>**Subculture guidelines:**<br><br>All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of $2x10^4$ cells/$cm^2$ is recommended.<br>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, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and 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** | 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** | Highly expressed in skeletal muscle, heart, liver and testis. |
| **Sequence similarities** | Belongs to the peptidase C54 family. |
| **Cellular localization** | Cytoplasm. |

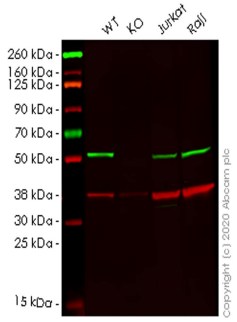
## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab267324 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: 52 kDa. |

## Images




Western blot - Human ATG4C knockout HEK293T cell line (ab267324)

**All lanes :** Anti-ATG4C antibody [EPR15001] (**ab183516**) at 1/1000 dilution

**Lane 1 :** Wild-type HEK293T cell lysate
**Lane 2 :** ATG4C knockout HEK293T cell lysate
**Lane 3 :** Jurkat cell lysate
**Lane 4 :** Raji 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

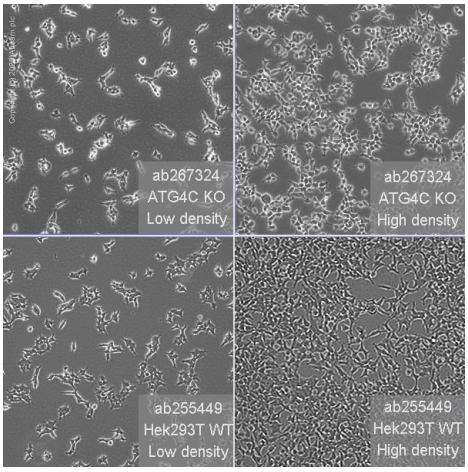
**Predicted band size:** 52 kDa
**Observed band size:** 52 kDa

**Lanes 1-4:** Merged signal (red and green). Green - **ab183516** observed at 52 kDa. Red - loading control **ab8245** observed at 36 kDa.

**ab183516** Anti-ATG4C antibody [EPR15001] was shown to specifically react with ATG4C in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab267324 (knockout cell lysate **ab257847**) was used. Wild-type and ATG4C knockout samples were subjected to SDS-PAGE. **ab183516** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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 ATG4C knockout HEK293T cell line (ab267324)

Homozygous: 1 bp insertion in exon5




Cell Culture - Human ATG4C knockout HEK293T cell line (ab267324)

Representative images of ATG4C knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.



## Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

## Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors