abcam

Product datasheet

Human ATG4B knockout HeLa cell lysate ab257364

4 Images

Overview

Product name	Human ATG4B knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon4 and 1 bp insertion in exon4.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. 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**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab260973 - Human ATG4B knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

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

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 Mainly expressed in the skeletal muscle, followed by brain, heart, liver and pancreas.

Sequence similarities Belongs to the peptidase C54 family.

Cellular localization Cytoplasm.

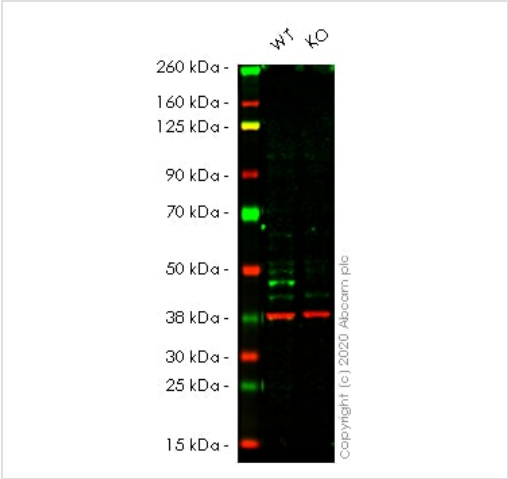
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257364 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

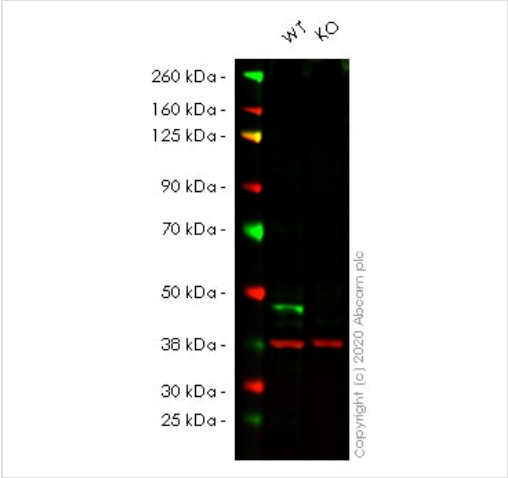
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 44 kDa.

Images



Western blot - Human ATG4B knockout HeLa cell lysate (ab257364)

Lane 1: Wild-type HeLa cell lysate 20 ug
Lane 2: ATG4B knockout HeLa cell lysate 20 ug
Lanes 1 - 2: Merged signal (red and green). Green - **ab199537** observed at 47 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab199537 was shown to react with ATG4B in wild-type HeLa cells in Western blot with loss of signal observed in ATG4B knockout cell line **ab260973** (ATG4B knockout cell lysate ab257364). Wild-type and ATG4B knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab199537** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human ATG4B knockout HeLa cell lysate (ab257364)

Lane 1: Wild-type HeLa cell lysate 20 ug
Lane 2: ATG4B knockout HeLa cell lysate 20 ug
Lanes 1 - 2: Merged signal (red and green). Green - **ab154843** observed at 47 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab154843 was shown to react with ATG4B in wild-type HeLa cells in Western blot with loss of signal observed in ATG4B knockout cell line **ab260973** (ATG4B knockout cell lysate ab257364). Wild-type and ATG4B knockout HEK293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab154843** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Sanger Sequencing - Human ATG4B knockout HeLa cell lysate (ab257364)

Allele-1: 1 bp deletion in exon4

Sanger Sequencing - Human ATG4B knockout HeLa cell lysate (ab257364)

Allele-2: 1 bp insertion in exon4

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