abcam

Product datasheet

Human ATG14 (ATG14L) knockout HeLa cell lysate ab258319

3 Images

Overview

Product name	Human ATG14 (ATG14L) 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, 17 bp deletion in exon 1 and Insertion of the selection cassette in exon 1.
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
ab260527 - Human ATG14 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 Required for both basal and inducible autophagy. Plays a role in autophagosome formation and MAP1LC3/LC3 conjugation to phosphatidylethanolamine. Promotes BECN1 translocation from the trans-Golgi network to autophagosomes. Enhances PIK3C3 activity in a BECN1-dependent manner.

Sequence similarities Belongs to the Barkor family.

Domain The coiled-coil domain is required for BECN1- and PIK3C3-binding and for autophagy.

Cellular localization Cytoplasm. Endoplasmic reticulum. Cytosolic under nutrient-rich conditions. Following autophagy stimuli, such as starvation or rapamycin induction, predominantly detected in cytoplasmic foci, identified as isolation membranes and autophagosomes.

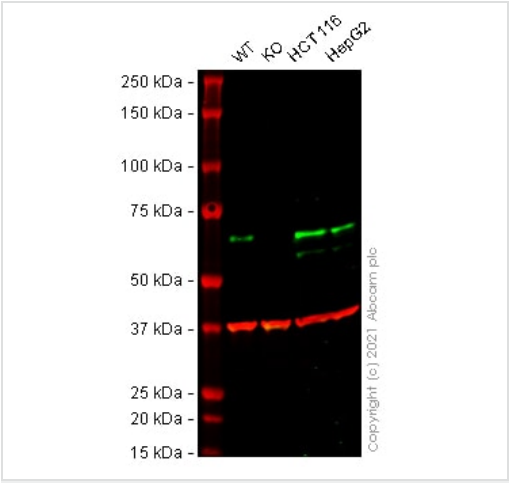
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258319 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.

Images



Western blot - Human ATG14 (ATG14L) knockout HeLa cell lysate (ab258319)

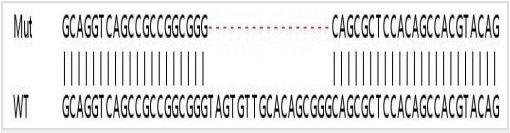
Lane 1: Wild-type HeLa cell lysate 20 µg

Lane 2: HeLa cell lysate 20 µg

Lane 3: HCT 116 cell lysate 20 µg

Lane 4: HepG2 cell lysate 20 µg

False colour image of Western blot: Anti-ATG14 antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, the antibody was shown to bind specifically to ATG14. A band was observed at 55 kDa in wild-type HeLa cell lysates with no signal observed at this size in ATG14 knockout cell line **ab264684** (knockout cell lysate ab258319). To generate this image, wild-type and ATG14 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 5 % 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 ATG14 knockout HeLa cell lysate (ab258319)

Allele-1: 17 bp deletion in exon 1

Sanger Sequencing - Human ATG14 knockout HeLa cell lysate (ab258319)

Allele-2: Insertion of the selection cassette in exon 1

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