abcam

Product datasheet

Human TNFAIP3 knockout HeLa cell lysate ab257112

3 Images

Overview

Product name	Human TNFAIP3 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 insertion in exon7.
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
ab262010 - Human TNFAIP3 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 Ubiquitin-editing enzyme that contains both ubiquitin ligase and deubiquitinase activities. Essential component of a ubiquitin-editing protein complex, comprising also RNF11, ITCH and TAX1BP1, that ensures the transient nature of inflammatory signaling pathways. Upon TNF stimulation, deubiquitinates 'Lys-63'-polyubiquitin chains on RIPK1 and catalyzes the formation of 'Lys-48'-polyubiquitin chains. This leads to RIPK1 proteasomal degradation and consequently termination of the TNF- or LPS-mediated activation of NF-kappa-B. In vitro able to deubiquitinate both 'Lys-48'- and 'Lys-63' polyubiquitin chains. Inhibitor of programmed cell death. Has a role in the function of the lymphoid system.

Sequence similarities Belongs to the peptidase C64 family.
Contains 7 A20-type zinc fingers.
Contains 1 OTU domain.

Domain The A20-type zinc fingers mediate the ubiquitin ligase activity.
The OTU domain mediates the deubiquitinase activity.

Cellular localization Cytoplasm. Nucleus.

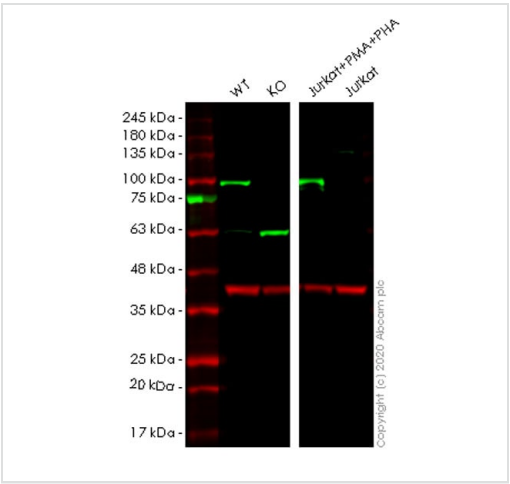
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257112 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: 89 kDa.

Images



Western blot - Human TNFAIP3 knockout HeLa cell lysate (ab257112)

Lane 1: Wild-type HeLa cell lysate (20 µg)

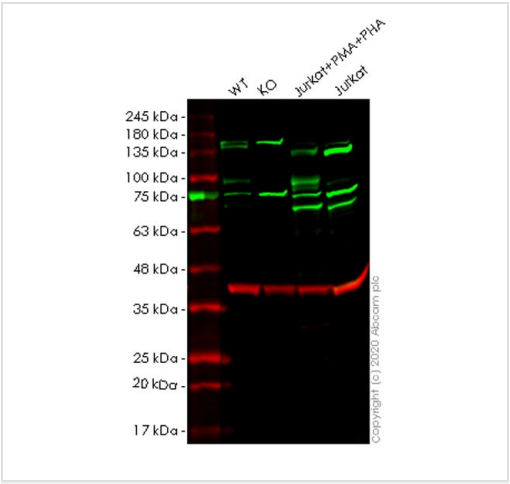
Lane 2: TNFAIP3 knockout HeLa cell lysate (20 µg)

Lane 3: Jurkat cell treated with 5ng/ml PMA for 48 hours and then treated with 2µg/ml PHA for 48 hours, whole cell lysate (20 µg)

Lane 4: Untreated Jurkat cell lysate (20 µg)

Lanes 1-4: Merged signal (red and green). Green - **ab92324** observed at 80 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab92324 Anti-TNFAIP3 antibody [EPR2663] was shown to specifically react with TNFAIP3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265983** (knockout cell lysate ab257112) was used. Wild-type and TNFAIP3 knockout samples were subjected to SDS-PAGE. **ab92324** 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 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Human TNFAIP3 knockout HeLa cell lysate (ab257112)

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Lane 2: TNFAIP3 knockout HeLa cell lysate (20 µg)

Lane 3: Jurkat cell treated with 5ng/ml PMA for 48 hours and then treated with 2µg/ml PHA for 48 hours, whole cell lysate (20 µg)

Lane 4: Untreated Jurkat cell lysate (20 µg)

Lanes 1-4: Merged signal (red and green). Green - **ab13597** observed at 80 kDa. Red - loading control, **ab181602** observed at 37 kDa.

ab13597 Anti-TNFAIP3 antibody [59A426] was shown to specifically react with TNFAIP3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265983** (knockout cell lysate ab257112) was used. Wild-type and TNFAIP3 knockout samples were subjected to SDS-PAGE. **ab13597** and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 10000 dilution for 1 hour at room

temperature before imaging.

Sanger Sequencing - Human TNFAIP3 knockout HeLa cell lysate (ab257112)

Homozygous: 1 bp insertion in exon7

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