abcam

Product datasheet

Human TRAF2 knockout HEK-293T cell lysate ab257759

3 Images

Overview

Product name	Human TRAF2 knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 7 bp deletion in exon 2.
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 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**.
Tested applications	**Suitable for:** WB

Properties

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

Components	1 kit
ab261059 - Human TRAF2 knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

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

Target

Function Regulates activation of NF-kappa-B and JNK and plays a central role in the regulation of cell survival and apoptosis. Required for normal antibody isotype switching from IgM to IgG. Has E3 ubiquitin-protein ligase activity and promotes 'Lys-63'-linked ubiquitination of target proteins, such as BIRC3, RIPK1 and TICAM1. Is an essential constituent of several E3 ubiquitin-protein ligase complexes, where it promotes the ubiquitination of target proteins by bringing them into contact with other E3 ubiquitin ligases. Regulates BIRC2 and BIRC3 protein levels by inhibiting their autoubiquitination and subsequent degradation; this does not depend on the TRAF2 RING-type zinc finger domain.

Pathway Protein modification; protein ubiquitination.

Sequence similarities Belongs to the TNF receptor-associated factor family. A subfamily.
Contains 1 MATH domain.
Contains 1 RING-type zinc finger.
Contains 2 TRAF-type zinc fingers.

Domain The coiled coil domain mediates homo- and hetero-oligomerization.
The MATH/TRAF domain binds to receptor cytoplasmic domains.
The RING-type zinc finger domain is essential for E3 ubiquitin-protein ligase activity. It is not essential for the stabilization of BIRC2, or for the ubiquitination of RIPK1 in response to TNFR1 signaling.

Post-translational modifications Phosphorylated at several serine residues within the first 128 amino acid residues.
Phosphorylated at Thr-117 in response to signaling via TNF and TNFRSF1A. Phosphorylation at Thr-117 is required for 'Lys-63'-linked polyubiquitination, but not for 'Lys-48'-linked polyubiquitination. Phosphorylation at Thr-117 is important for interaction with IKKA and IKKB, activation of IKK and subsequent activation of NF-kappa-B.
Undergoes both 'Lys-48'-linked and 'Lys-63'-linked polyubiquitination. Polyubiquitinated via 'Lys-63'-linked ubiquitin in response to TNF signaling; this requires prior phosphorylation at Thr-117. 'Lys-63'-linked polyubiquitination promotes TRAF2-mediated activation of NF-kappa-B. Can be polyubiquitinated at several Lys residues via 'Lys-48'-linked ubiquitin chains in response to TNF signaling, leading to proteasomal degradation. Autoubiquitinated, leading to its subsequent proteasomal degradation. Polyubiquitinated by BIRC2 and SIAH2, leading to its subsequent proteasomal degradation. Deubiquitinated by CYLD, a protease that specifically cleaves 'Lys-63'-linked polyubiquitin chains.

Cellular localization Cytoplasm.

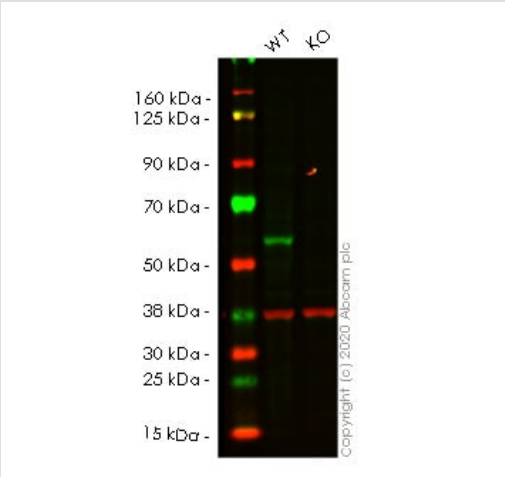
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257759 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: 55 kDa.

Images



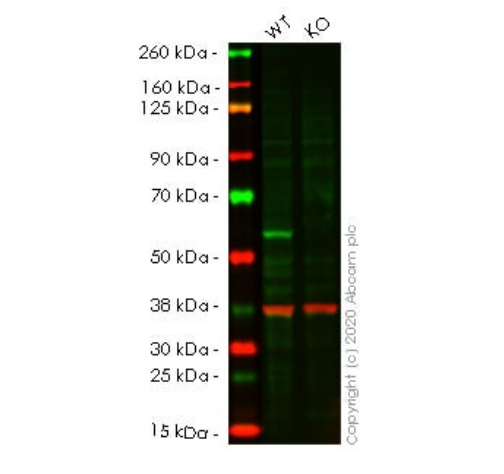
Western blot - Human TRAF2 knockout HEK293T cell lysate (ab257759)

Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: TRAF2 knockout HEK-293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab126758** observed at 55 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab126758 Anti-TRAF2 antibody [EPR6048] was shown to specifically react with TRAF2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266060** (knockout cell lysate ab257759) was used. Wild-type and TRAF2 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab126758** 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.



Western blot - Human TRAF2 knockout HEK293T cell lysate (ab257759)

Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: TRAF2 knockout HEK-293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab167163** observed at 55 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab167163 Anti-TRAF2 antibody [EPR7064] was shown to specifically react with TRAF2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266060** (knockout cell lysate ab257759) was used. Wild-type and TRAF2 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab167163** 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 TRAF2 knockout HEK293T cell lysate (ab257759)

Homozygous: 7 bp deletion in exon 2

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

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