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Product datasheet

Human TRAF2 knockout HEK-293T cell line ab266060

4 Images

Overview

Product name	Human TRAF2 knockout HEK-293T cell line
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)
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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. 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 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**.

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
Antibiotic resistance	Puromycin 1.00µg/ml
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	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.

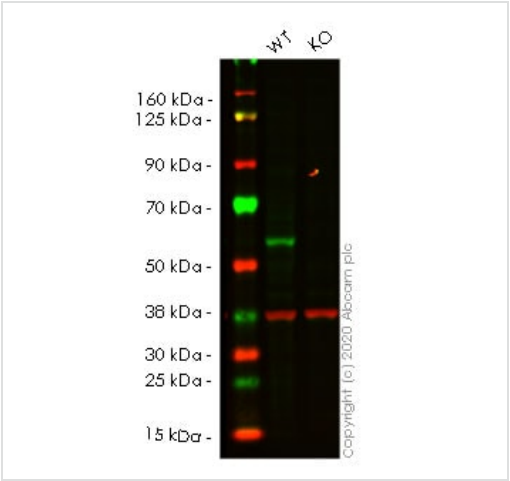
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266060 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 line (ab266060)

All lanes : Anti-TRAF2 antibody [EPR6048] (**ab126758**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : TRAF2 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

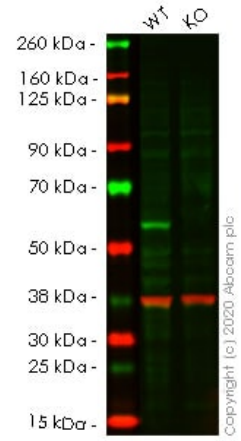
Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 55 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab126758** observed at 55 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab126758 was shown to 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 HEK-293T and TRAF2 knockout HEK-293T cell lysates 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**) overnight at 4°C at a 1 in 1000 dilution and a 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 line (ab266060)

All lanes : Anti-TRAF2 antibody [EPR7064] (**ab167163**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : TRAF2 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

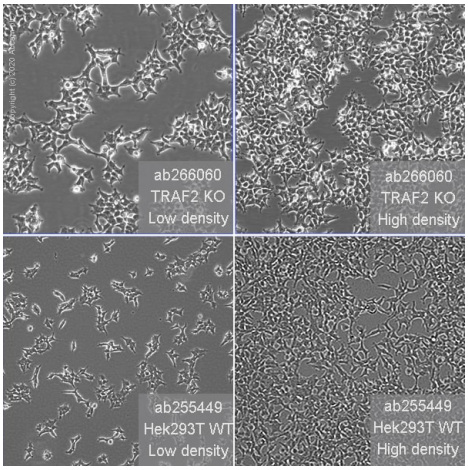
Predicted band size: 55 kDa
Observed band size: 55 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab167163** observed at 55 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab167163 was shown to 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 HEK-293T and TRAF2 knockout HEK-293T cell lysates 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**) overnight at 4°C at a 1 in 1000 dilution and a 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 line (ab266060)

Homozygous: 7 bp deletion in exon 2



Cell Culture - Human TRAF2 knockout HEK293T cell line (ab266060)

Representative images TRAF2 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 a EVOS M5000 microscope.

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