

Product datasheet

Human IKBKG (IKK gamma/NEMO) knockout HEK-293T cell line ab266674

4 Images

Overview

Product name	Human IKBKG (IKK gamma/NEMO) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 13 bp deletion in exon 2 and 1 bp insertion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.</p> <p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p>

All seeding densities should be based on cell counts gained by established methods.

A guide seeding density of 2×10^4 cells/cm² 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.

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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	Regulatory subunit of the IKK core complex which phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. Also considered to be a mediator for TAX activation of NF-kappa-B. Could be implicated in NF-kappa-B-mediated protection from cytokine toxicity (By similarity). Essential for viral activation of IRF3.
Tissue specificity	Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
Involvement in disease	<p>Defects in IKBKG are the cause of ectodermal dysplasia anhidrotic with immunodeficiency X-linked (EDAID) [MIM:300291]; also known as hypohidrotic ectodermal dysplasia with immunodeficiency (HED-ID). Is a form of ectoderma dysplasia, a heterogeneous group of disorders due to abnormal development of two or more ectodermal structures. Characterized by absence of sweat glands, sparse scalp hair, rare conical teeth and immunological abnormalities resulting in severe infectious diseases.</p> <p>Defects in IKBKG are the cause of ectodermal dysplasia anhidrotic with immunodeficiency-osteopetrosis-lymphedema (OLEDAID) [MIM:300301].</p> <p>Defects in IKBKG are a cause of immunodeficiency NEMO-related without anhidrotic ectodermal dysplasia (NEMOID) [MIM:300584]; also called immunodeficiency without anhidrotic ectodermal dysplasia, isolated immunodeficiency or pure immunodeficiency. Patients manifest immunodeficiency not associated with other abnormalities, and resulting in increased infection susceptibility. Patients suffer from multiple episodes of infectious diseases.</p> <p>Defects in IKBKG are the cause of susceptibility to X-linked familial atypical micobacteriosis type</p>

1 (AMCBX1) [MIM:300636]; also known as X-linked disseminated atypical mycobacterial infection type 1 or X-linked susceptibility to mycobacterial disease type 1. AMCBX1 is the X-linked recessive form of mendelian susceptibility to mycobacterial disease (MSMD). MSMD is a congenital syndrome resulting in predisposition to clinical disease caused by weakly virulent mycobacterial species, such as bacillus Calmette-Guerin vaccines and non-tuberculous, environmental mycobacteria. Patients are also susceptible to the more virulent species *Mycobacterium tuberculosis*.

Defects in IKBKG are the cause of recurrent isolated invasive pneumococcal disease type 2 (IPD2) [MIM:300640]. Recurrent invasive pneumococcal disease (IPD) is defined as two episodes of IPD occurring at least 1 month apart, whether caused by the same or different serotypes or strains. Recurrent IPD occurs in at least 2% of patients in most series, making IPD the most important known risk factor for subsequent IPD.

Defects in IKBKG are the cause of incontinentia pigmenti (IP) [MIM:308300]; formerly designed familial incontinentia pigmenti type II (IP2). IP is a genodermatosis usually prenatally lethal in males. In affected females, it causes abnormalities of the skin, hair, eyes, nails, teeth, skeleton, heart, and central nervous system. The prominent skin signs occur in four classic cutaneous stages: perinatal inflammatory vesicles, verrucous patches, a distinctive pattern of hyperpigmentation and dermal scarring.

Sequence similarities

Contains 1 C2HC-type zinc finger.

Domain

The leucine-zipper domain and the C2HC-type zinc-finger are essential for polyubiquitin binding and for the activation of IRF3.

Post-translational modifications

Phosphorylation at Ser-68 attenuates aminoterminal homodimerization.

Polyubiquitinated on Lys-285 through 'Lys-63'; the ubiquitination is mediated by NOD2 and RIPK2 and probably plays a role in signaling by facilitating interactions with ubiquitin domain-containing proteins and activates the NF-kappa-B pathway. Polyubiquitinated on Lys-399 through 'Lys-63'; the ubiquitination is mediated by BCL10, MALT1 and TRAF6 and probably plays a role in signaling by facilitating interactions with ubiquitin domain-containing proteins and activates the NF-kappa-B pathway. Monoubiquitinated on Lys-277 and Lys-309; promotes nuclear export. Linear polyubiquitinated on Lys-285; the head-to-tail polyubiquitination is mediated by the LUBAC complex. Linear polyubiquitinated on Lys-309; the head-to-tail polyubiquitination is mediated by the LUBAC complex.

Sumoylated on Lys-277 and Lys-309 by SUMO1; the modification results in phosphorylation of Ser-85 by ATM leading to a replacement of the sumoylation by mono-ubiquitination on these residues.

Cellular localization

Cytoplasm. Nucleus. Sumoylated NEMO accumulates in the nucleus in response to genotoxic stress.

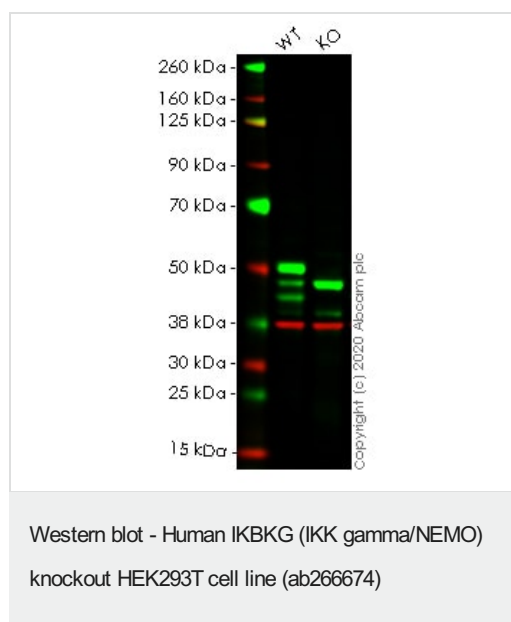
Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab266674 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: 48 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.



All lanes : Anti-IKK gamma/NEMO antibody [EPR14660] ([ab188569](#)) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate

Lane 2 : IKBKG knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

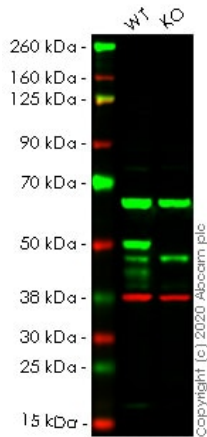
Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 48 kDa

Lanes 1- 2: Merged signal (red and green). Green - [ab188569](#) observed at 48 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

[ab188569](#) was shown to react with IKK gamma/NEMO in wild-type HEK-293T cells in western blot. The band observed in knockout cell line ab266674 (knockout cell lysate [ab257153](#)) lane below 48kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HEK-293T and IKBKG 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. [ab188569](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated 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 IKBKG (IKK gamma/NEMO) knockout HEK293T cell line (ab266674)

All lanes : Anti-IKK gamma/NEMO antibody [EPR16629] ([ab178872](#)) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate

Lane 2 : IKBKG knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 48 kDa

Lanes 1- 2: Merged signal (red and green). Green - [ab178872](#) observed at 48 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

[ab178872](#) was shown to react with IKK gamma/NEMO in wild-type HEK-293T cells in western blot. The band observed in knockout cell line ab266674 (knockout cell lysate [ab257153](#)) lane below 48kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HEK-293T and IKBKG 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. [ab178872](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated 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.

Mut	GACTCTTCGCCCAGTACGTCTGATCTGCT-----TGGGCTGCACCATCTCA
WT	GACTCTTCGCCCAGTACGTCTGATCTGCTGCCGGGCCACCACTGGGCTGCACCATCTCA
Sanger Sequencing - Human IKBKG knockout	
HEK293T cell line (ab266674)	

Allele-1: 13 bp deletion in exon 2

Mut	GACTCTTCGCCCAGTACGTCTGATCTGCTGCCGGGCCACCACTGGGCTGCACCATCTC
WT	GACTCTTCGCCCAGTACGTCTGATCTGCTGCCGGGCCACCACTGGGCTGCACCATCTC
Sanger Sequencing - Human IKBKG knockout	
HEK293T cell line (ab266674)	

Allele-2: 1 bp insertion in exon 2.

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