abcam

Product datasheet

Anti-IKK gamma/NEMO antibody [EPR16629] ab178872

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 16 References 11 Images

Overview

Product name	Anti-IKK gamma/NEMO antibody [EPR16629]
Description	Rabbit monoclonal [EPR16629] to IKK gamma/NEMO
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human fetal brain and kidney tissue lysates; Human colon cancer tissue lysate; HeLa, K562, Jurkat, HEK293T C6, RAW 264.7, PC-12 and NIH/3T3 cell lysates; Mouse brain, heart, kidney and spleen tissue lysates; Rat brain, heart, kidney and spleen tissue lysates. IHC-P: Human colonic adenocarcinoma, rat colon. ICC/IF: HeLa, NIH/3T3
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16629

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab178872 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	★★★★★ (1)	1/5000. Detects a band of approximately 37-60 kDa (predicted molecular weight: 48 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/250.
IP		1/50.

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 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.
Defects in IKBKG are the cause of ectodermal dysplasia anhidrotic with immunodeficiency-osteopetrosis-lymphedema (OLEDAID) [MIM:300301].
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.
Defects in IKBKG are the cause of susceptibility to X-linked familial atypical micobacteriosis type 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.

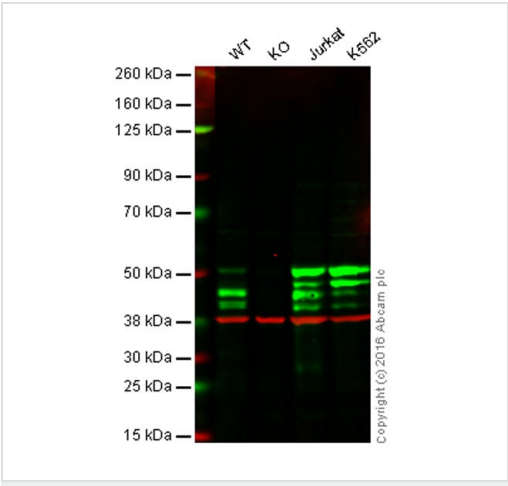
Post-translational modifications

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.

Images

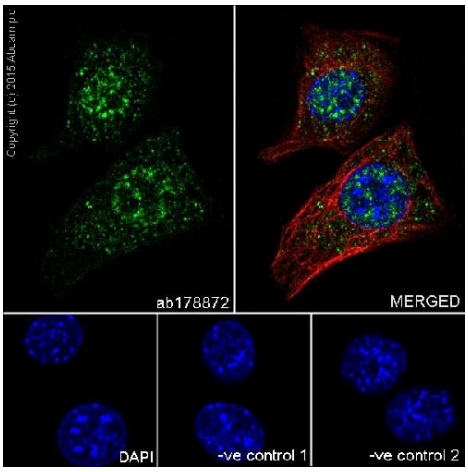


Western blot - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: IKK gamma/NEMO knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: K562 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab178872 observed at 40, 45, 50 kDa. Red - loading control, ab8245, observed at 37 kDa.

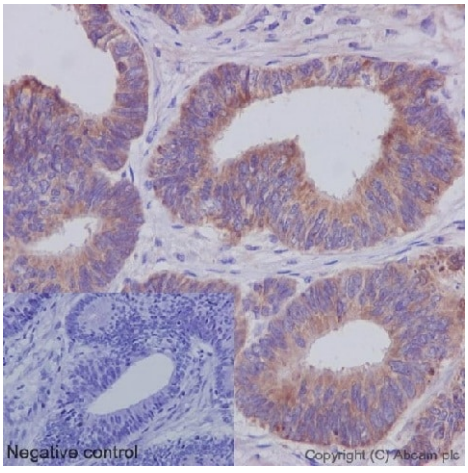
ab178872 was shown to react with IKK gamma in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when IKK gamma/NEMO knockout samples were examined. Wild-type and IKK gamma/NEMO knockout samples were subjected to SDS-PAGE. ab178872 and ab8245 (loading control to GAPDH) were diluted at 1/5000 and 1/10,000 respectively and incubated overnight at 4°C. 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 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

Immunofluorescence analysis of 4% paraformaldehyde fixed, 0.1% Triton X-100 permeabilized NIH/3T3 cells (Mouse embyro fibroblast cells) labeling IKK gamma/NEMO (green) with ab178872 at 1/250 dilution showing cytoplasm and nucleus staining. Secondary ab: Goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/200 dilution. Counter stain is labeling tubulin (red) with ab7291 at 1/500 dilution with secondary antibody Goat anti-Mouse AlexaFluor® 594 (ab150120) at 1/400 dilution. DAPI stains the nucleus in blue. -ve control 1 is ab178872 at 1/250 dilution, ab150120 at 1/400 dilution. -ve control 2 is ab7291 at 1/500 dilution, ab150077 at 1/200 dilution.

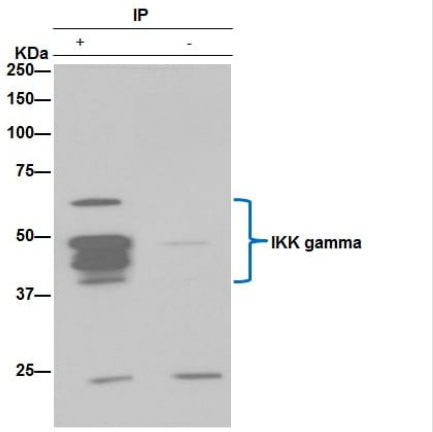


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

Immunohistochemical analysis of paraffin embedded human colonic adenocarcinoma tissue labeling IKK gamma/NEMO with ab178872 at 1/100 dilution followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasmic staining on colonic adenocarcinoma is observed.

Negative control: Using PBS instead of primary ab, secondary ab ImmunoHistoprobe (Ready to use) HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

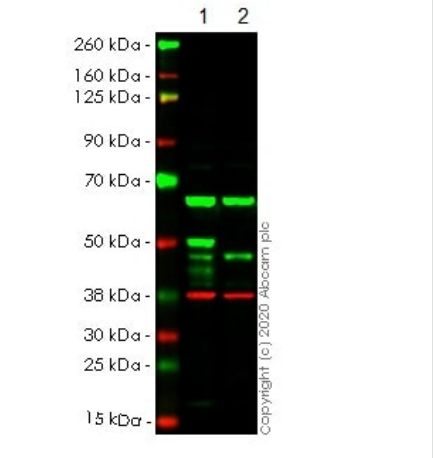


Immunoprecipitation - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

IKK gamma/NEMO was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell extract with ab178872 at 1/50 dilution. Western blot was performed of the immunoprecipitate using ab178872 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution. Left lane: HeLa whole cell extract. Right lane: PBS instead of HeLa whole cell extract.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : IKBKG CRISPR/Cas9 edited HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

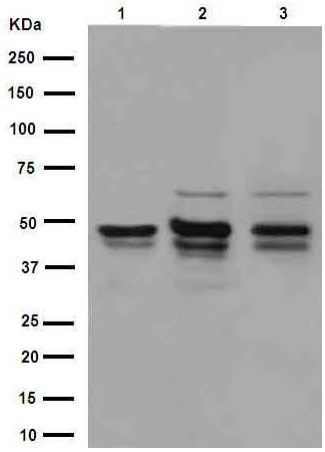
Performed under reducing conditions.

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 CRISPR/Cas9 edited cell line ab266674 (CRISPR/Cas9 edited 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 CRISPR/Cas9 edited 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.



Western blot - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

All lanes : Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872) at 1/5000 dilution

Lane 1 : Human fetal brain
Lane 2 : Human fetal kidney
Lane 3 : Human colon cancer

Lysates/proteins at 10 µg per lane.

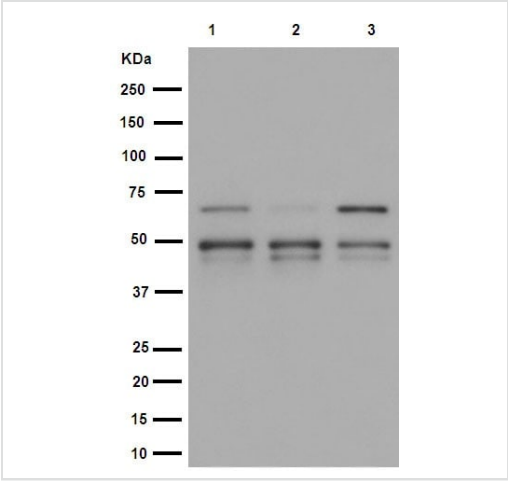
Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 48 kDa
Additional bands at: 37,48,56 kDa (possible isoform)

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM /TBST

ab178872 could recognize 3 isoforms with the predicted MWs of 37KDa, 56KDa and 48KDa, respectively.



Western blot - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

All lanes : Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872) at 1/20000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates
Lane 2 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysates
Lane 3 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates

Lysates/proteins at 20 µg per lane.

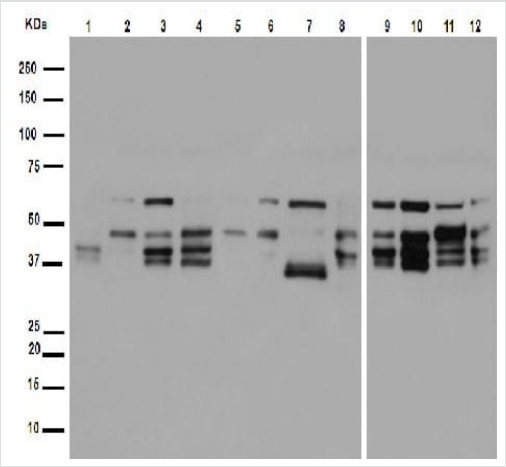
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 48 kDa
Observed band size: 46-60 kDa

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM /TBST

ab178872 could recognize 3 isoforms with the predicted MWs of 37KDa, 56KDa and 48KDa, respectively.



Western blot - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

All lanes : Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872) at 1/20000 dilution

Lane 1 : Mouse brain
Lane 2 : Mouse heart
Lane 3 : Mouse kidney
Lane 4 : Mouse spleen
Lane 5 : Rat brain
Lane 6 : Rat heart
Lane 7 : Rat kidney
Lane 8 : Rat spleen
Lane 9 : C6 (Rat glial tumor cells) whole cell lysates
Lane 10 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysates
Lane 11 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates
Lane 12 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysates

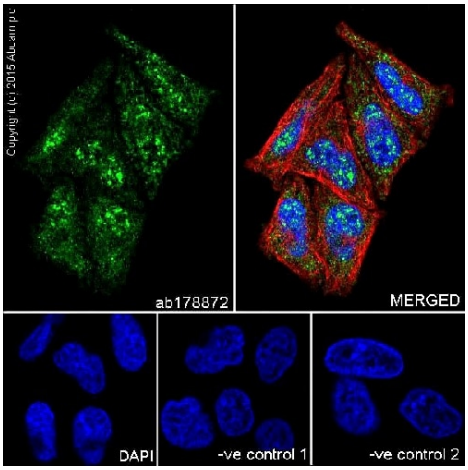
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 48 kDa
Observed band size: 37-60 kDa

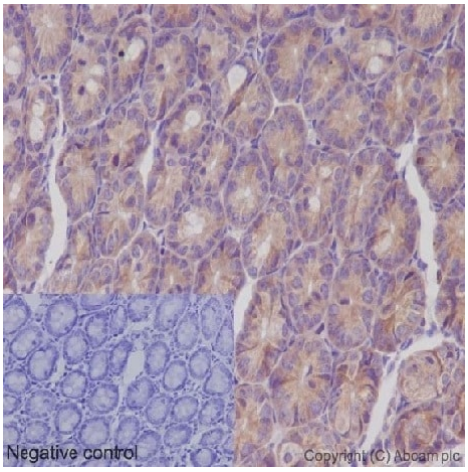
Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM /TBST



Immunocytochemistry/ Immunofluorescence - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

Immunofluorescence analysis of 4% paraformaldehyde fixed, 0.1% Triton X-100 permeabilized HeLa cells (Human epithelial cells from cervix adenocarcinoma) labeling IKK gamma/NEMO (green) with ab178872 at 1/250 dilution showing cytoplasm and nucleus staining. Secondary ab: Goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/200 dilution. Counter stain is labeling tubulin (red) with ab7291 at 1/500 dilution with secondary antibody Goat anti-Mouse AlexaFluor® 594 (ab150120) at 1/400 dilution. DAPI stains the nucleus in blue. -ve control 1 is ab178872 at 1/250 dilution, ab150120 at 1/400 dilution. -ve control 2 is ab7291 at 1/500 dilution, ab150077 at 1/200 dilution.

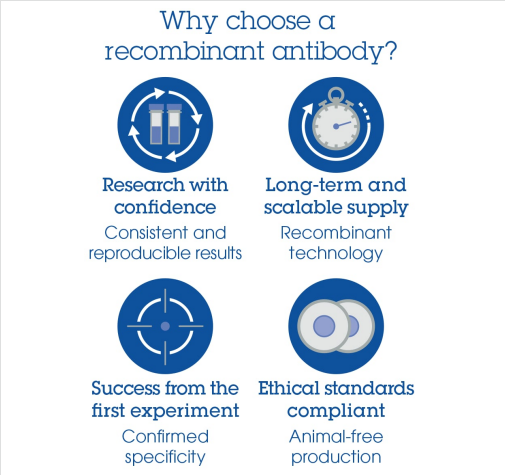


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK gamma/NEMO antibody [EPR16629] (ab178872)

Immunohistochemical analysis of paraffin embedded Rat colon tissue labeling IKK gamma/NEMO with ab178872 at 1/100 dilution followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Counter stain is Hematoxylin. Cytoplasm staining on epithelial cells of rat colon is observed.

Negative control: Using PBS instead of primary ab, secondary ab as above.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-IKK gamma/NEMO antibody [EPR16629]

(ab178872)

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