abcam

Product datasheet

# Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free ab230832

KO VALIDATED Recombinant RabMAb

1 Abreviews 8 Images

## Overview

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|---|---|
| **Product name** | Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR16629] to IKK gamma/NEMO - BSA and Azide free |
| **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, Human fetal kidney, Human colon cancer, HEK293 whole cell lysates, HeLa whole cell lysates, K562 whole cell lysates, Jurkat whole cell lysates. Mouse brain, heart, kidney and spleen. Rat brain, heart, kidney and spleen. C6 whole cell lysates, RAW 264.7 whole cell lysates, PC-12 whole cell lysates, NIH/3T3 whole cell lysates. IHC-P: Human colonic adenocarcinoma, rat colon. ICC/IF: HeLa, NIH/3T3 |
| **General notes** | ab230832 is the carrier-free version of **ab178872**.<br><br>Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.<br><br>Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.<br><br>This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. |

## Properties

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| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |

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| **Storage buffer** | pH: 7.2<br>Constituent: PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR16629 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab230832 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. Detects a band of approximately 37-60 kDa (predicted molecular weight: 48 kDa). |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **ICC/IF** | | Use at an assay dependent concentration. |
| **IP** | | Use at an assay dependent concentration. |

## Target

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| **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.<br>Defects in IKBKG are the cause of ectodermal dysplasia anhidrotic with immunodeficiency-osteopetrosis-lymphedema (OLEDAID) [MIM:300301].<br>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. |

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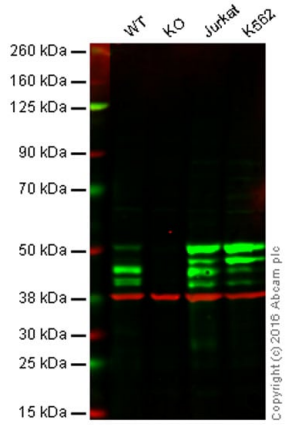
| | Defects in IKBKG are the cause of susceptibility to X-linked familial atypical micobacteriosis type 1 (AMCBX1) [MIM:300636]; also known as X-linked disseminated atypical mycobacterial infection type 1 or X-linked susceptibility to mycobacterial disease type 1. AMCBX1 is the X-linked recessive form 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.<br>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.<br>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.<br>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.<br>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

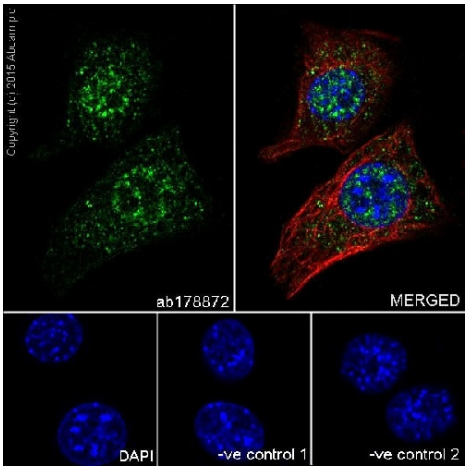



Western blot - Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free (ab230832)

This WB data was generated using the same anti-IKK gamma antibody clone, EPR16629, in a different buffer formulation (cat# **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.

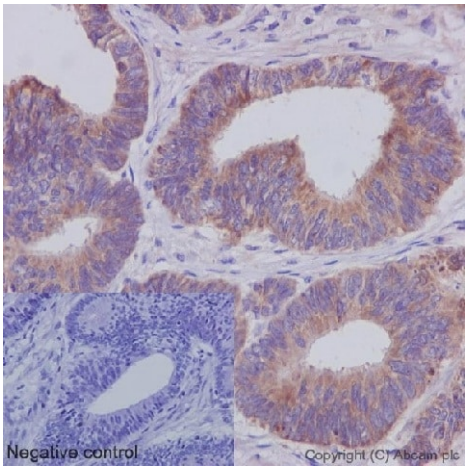
**ab178872** was shown to react with IKK gamma in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when IKK gamma/NEMO knockout samples were examined. Wild-type and IKK gamma/NEMO knockout samples were subjected to SDS-PAGE. **ab178872** and **ab8245** (loading control 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] - BSA and Azide free (ab230832)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178872**).



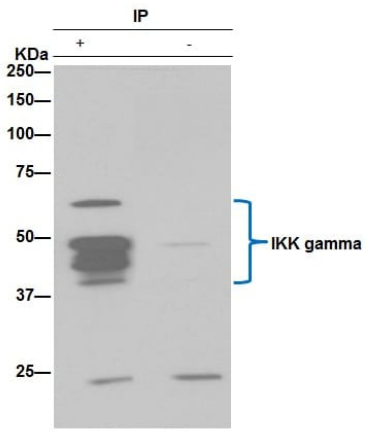

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free (ab230832)

This IHC data was generated using the same anti-IKK gamma/NEMO antibody clone, EPR16629, in a different buffer formulation (cat# **ab178872**).

Immunohistochemical analysis of paraffin embedded human colonic adenocarcinoma tissue labeling IKK gamma 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.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



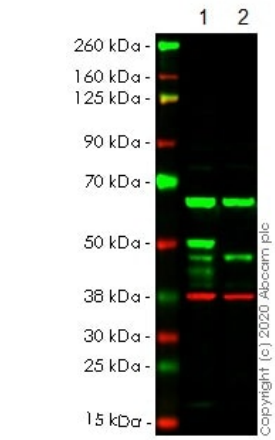

Immunoprecipitation - Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free (ab230832)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178872**).




Western blot - Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free (ab230832)

**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.

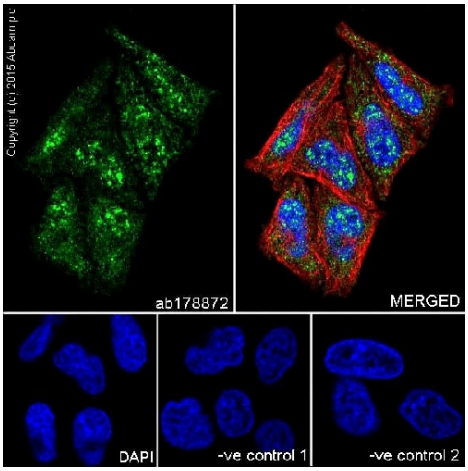
**Predicted band size:** 48 kDa
**Observed band size:** 48 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab178872**).

**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

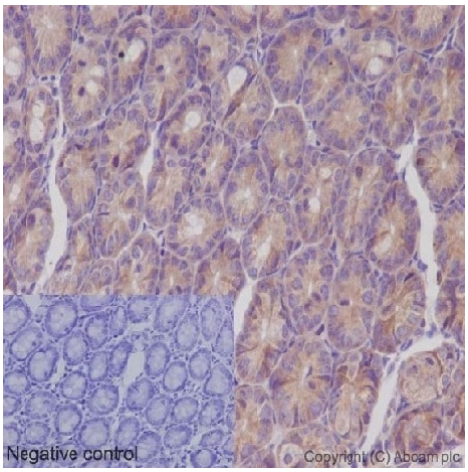
milk. **ab178872** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.




Immunocytochemistry/ Immunofluorescence - Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free (ab230832)

This ICC data was generated using the same anti-IKK gamma/NEMO antibody clone, EPR16629, in a different buffer formulation (cat# **ab178872**).

Immunofluorescence analysis of 4% paraformaldehyde fixed, 0.1% Triton X-100 permeabilized HeLa cells (Human epithelial cells from cervix adenocarcinoma) labeling IKK gamma (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.



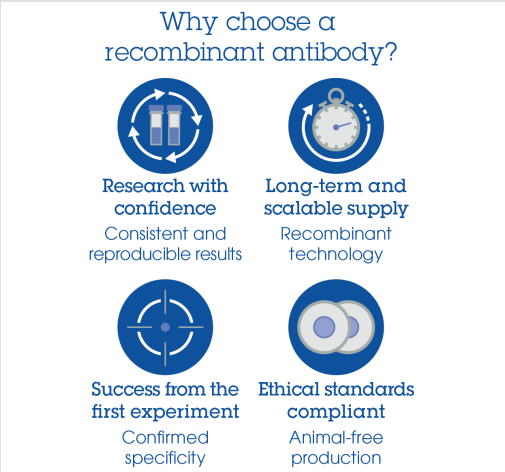

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free (ab230832)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178872**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.




Anti-IKK gamma/NEMO antibody [EPR16629] - BSA and Azide free (ab230832)

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