abcam

Product datasheet

Anti-IKK beta antibody [EPR6043] - BSA and Azide free ab171364



Recombinant

RabMAb

6 Images

Overview

Product name Anti-IKK beta antibody [EPR6043] - BSA and Azide free

Description Rabbit monoclonal [EPR6043] to IKK beta - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Unsuitable for: Flow Cyt (Intra),ICC/IF or IP

Species reactivity Reacts with: Mouse, Human

Predicted to work with: Rat

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

General notes ab171364 is the carrier-free version of **ab124957**.

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.

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.

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.

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.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to ${\hbox{\bf RabMAb}}^{\hbox{\bf @}}$ patents.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

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Storage buffer Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR6043

Isotype IgG

Applications

The Abpromise quarantee Our Abpromise quarantee covers the use of ab171364 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 85 kDa (predicted molecular weight: 87 kDa). Can be blocked with ab154148
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. (Heat to 98°C, allow to cool for 10-20 minutes)

Application notes Is unsuitable for Flow Cyt (Intra),ICC/IF or IP.

Target

Function Acts as part of the IKK complex in the conventional pathway of NF-kappa-B activation and

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 phosphorylates NCOA3.

Tissue specificity Highly expressed in heart, placenta, skeletal muscle, kidney, pancreas, spleen, thymus, prostate,

testis and peripheral blood.

Sequence similaritiesBelongs to the protein kinase superfamily. Ser/Thr protein kinase family. I-kappa-B kinase

subfamily.

Contains 1 protein kinase domain.

Post-translational modifications

Upon cytokine stimulation, phosphorylated on Ser-177 and Ser-181 by MEKK1 and/or

MAP3K14/NIK; which enhances activity. Once activated, autophosphorylates on the C-terminal serine cluster; which decreases activity and prevents prolonged activation of the inflammatory

response.

Acetylation of Thr-180 by Yersinia yopJ prevents phosphorylation and activation, thus blocking the

I-kappa-B pathway.

Ubiquitinated. Monoubiquitination involves TRIM21 that leads to inhibition of Tax-induced NF-

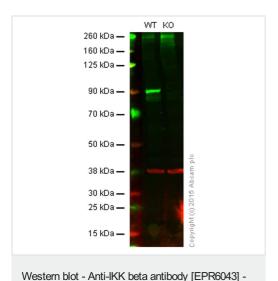
kappa-B signaling. According to PubMed:19675099, 'Ser-163' does not serve as a

monoubiquitination site. According to PubMed:16267042, ubiquitination on 'Ser-163' modulates phosphorylation on C-terminal serine residues. Monoubiquitination by TRIM21 is dirupted by

Yersinia yopJ.

Cellular localizationCytoplasm. Membrane raft. Colocalized with DPP4 in membrane rafts.

Images



BSA and Azide free (ab171364)

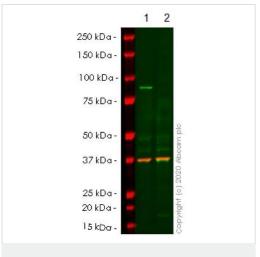
This WB data was generated using the same anti-IKK beta antibody clone [EPR6043] in a different buffer formulation (cat# ab124957).

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: IKK beta knockout HAP1 cell lysate (20 µg)

Lanes 1 and 2: Merged signal (red and green). Green - <u>ab124957</u> observed at 90 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab124957 was shown to specifically react with IKK beta when IKK beta knockout samples were used. Wild-type and IKK beta knockout samples were subjected to SDS-PAGE. ab124957 and ab8245 (loading control to GAPDH) were diluted 1/500 and 1/2000 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 h at room temperature before imaging.



Western blot - Anti-IKK beta antibody [EPR6043] -

BSA and Azide free (ab171364)

All lanes : Anti-IKK beta antibody [EPR6043] (ab124957) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: IKBKB CRISPR/Cas9 edited HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

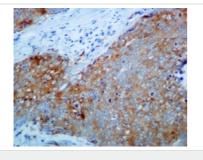
Predicted band size: 87 kDa **Observed band size:** 90 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab124957).

Lanes 1-2: Merged signal (red and green). Green - <u>ab124957</u> observed at 90 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

ab124957 was shown to react with IKK beta in wild-type HeLa cells

in western blot. The band observed in CRISPR/Cas9 edited cell line ab264847 (CRISPR/Cas9 edited cell lysate ab264847 (CRISPR/Cas9 edited cell lysate ab267628) lane below 90kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and IKBKB CRISPR/Cas9 edited HeLa 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. ab124957 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



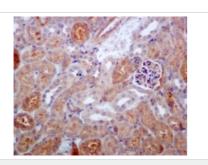
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKK beta antibody

[EPR6043] - BSA and Azide free (ab171364)

ab124957, at 1/100 dilution staining IKK beta in paraffin-embedded Human cervix carcinoma tissue, by Immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab124957).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



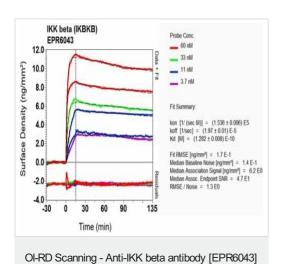
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKK beta antibody

[EPR6043] - BSA and Azide free (ab171364)

<u>ab124957</u>, at 1/100 dilution staining IKK beta in paraffin-embedded Mouse kidney tissue, by Immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124957**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

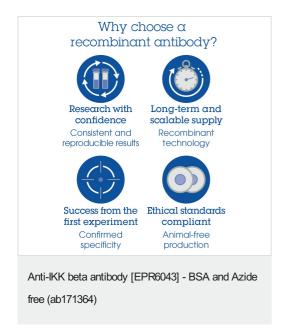


- BSA and Azide free (ab171364)

Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab124957</u>).



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