abcam

Product datasheet

Anti-IKB beta antibody [EPR5037] ab109509





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Overview

Product name Anti-IKB beta antibody [EPR5037]

Description Rabbit monoclonal [EPR5037] to IKB beta

Host species Rabbit

Tested applications Suitable for: ICC/IF, Flow Cyt (Intra), WB, IP, IHC-P

Species reactivity Reacts with: Human

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

Positive control WB: Jurkat, HeLa, THP-1, and MCF7 cell lysates. IP: HeLa cells. IHC-P: Human kidney tissue.

ICC/IF: Jurkat cells. Flow Cyt (intra): Jurkat cells.

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal **EPR5037** Clone number

Isotype lgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab109509 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
ICC/IF		1/50. For unpurified use at 1/100 - 1/250.
Flow Cyt (Intra)		1/20.
WB	★★★★ (2)	1/1000 - 1/10000. Detects a band of approximately 48 kDa (predicted molecular weight: 37 kDa).
IP		1/10 - 1/100.
IHC-P		1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/100 - 1/250.

Target

Function Inhibits NF-kappa-B by complexing with and trapping it in the cytoplasm. However, the

> unphosphorylated form resynthesized after cell stimulation is able to bind NF-kappa-B allowing its transport to the nucleus and protecting it to further NFKBIA-dependent inactivation. Association with inhibitor kappa B-interacting NKIRAS1 and NKIRAS2 prevent its phosphorylation rendering it

more resistant to degradation, explaining its slower degradation.

Tissue specificity Expressed in all tissues examined.

Sequence similarities Belongs to the NF-kappa-B inhibitor family.

Contains 6 ANK repeats.

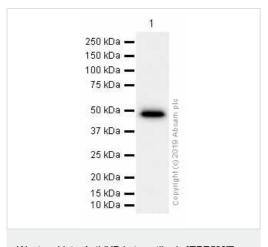
Post-translational modifications

Phosphorylated by RPS6KA1; followed by degradation. Interaction with NKIRAS1 and NKIRAS2

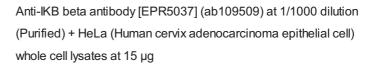
probably prevents phosphorylation.

Cellular localization Cytoplasm. Nucleus.

Images



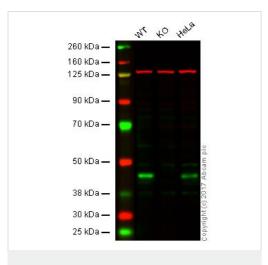
Western blot - Anti-IKB beta antibody [EPR5037] (ab109509)



Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

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



Western blot - Anti-IKB beta antibody [EPR5037] (ab109509)

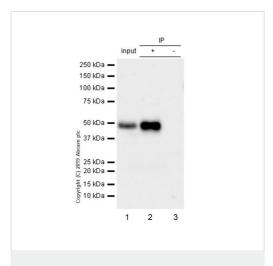
Lane 1: Wild type HAP1 whole cell lysate (20 µg)

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

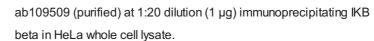
Lane 3: HeLa whole cell lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - unpurified ab109509 observed at 45 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab109509 was shown to specifically react with IKB beta in wild type cells as signal was lost in IKB beta knockout cells. Wild-type and IKB beta knockout samples were subjected to SDS-PAGE. ab109509 and ab18058 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-IKB beta antibody [EPR5037] (ab109509)



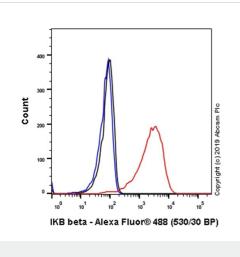
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

Lane 2 (+): ab109509 & HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab109509 in HeLa whole cell lysate

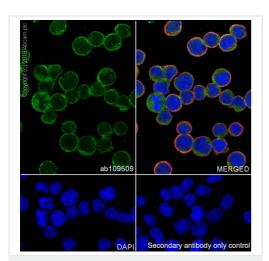
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



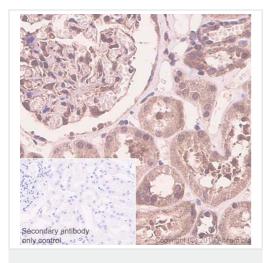
Flow Cytometry (Intracellular) - Anti-IKB beta antibody [EPR5037] (ab109509)

Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling IKB beta with purified ab109509 at 1/20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



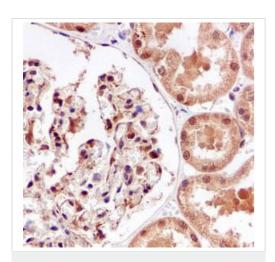
Immunocytochemistry/ Immunofluorescence - Anti-IKB beta antibody [EPR5037] (ab109509)

Immunocytochemistry/ Immunofluorescence analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling IKB beta with purified ab109509 at 1:50 dilution (2.0 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μ g/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKB beta antibody
[EPR5037] (ab109509)

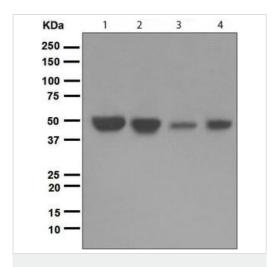
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue sections labeling IKB beta with purified ab109509 at 1:500 dilution (0.20 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKB beta antibody
[EPR5037] (ab109509)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue using unpurified ab109509 at a dilution of 1/100.

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-IKB beta antibody [EPR5037] (ab109509)

All lanes : Anti-IKB beta antibody [EPR5037] (ab109509) at 1/1000 dilution (Unpurified)

Lane 1 : Jurkat cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : THP-1 cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 37 kDa **Observed band size:** 48 kDa



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