abcam

Product datasheet

Anti-IKK alpha antibody [Y463] - BSA and Azide free ab169743

KO VALIDATED Recombinant RabMAb

3 References 8 Images

Overview

Product name	Anti-IKK alpha antibody [Y463] - BSA and Azide free
Description	Rabbit monoclonal [Y463] to IKK alpha - BSA and Azide free
Host species	Rabbit
Specificity	The rat recommendation is based on the WB results. We do not guarantee IHC-P for rat.
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1-WT; Daudi,RAW 264.7 and C6 whole cell lysate. Mouse and rat kidney lysate. IP: HeLa cell lysate; IHC-P: Human ovarian cancer tissue and Mouse kidney tissue; Flow Cyt (intra): HAP1 wildtype and Daudi cells.
General notes	ab169743 is the carrier-free version of **ab32041**. 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. 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. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y463
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab169743 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

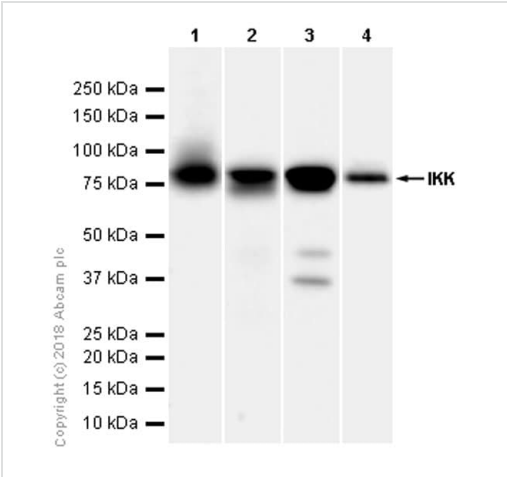
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 88 kDa (predicted molecular weight: 85 kDa).

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. As part of the non-canonical pathway of NF-kappa-B activation, the MAP3K14-activated CHUK/IKKA homodimer phosphorylates NFKB2/p100 associated with RelB, inducing its proteolytic processing to NFKB2/p52 and the formation of NF-kappa-B RelB-p52 complexes. Also phosphorylates NCOA3. Phosphorylates 'Ser-10' of histone H3 at NF-kappa-B-regulated promoters during inflammatory responses triggered by cytokines.
Tissue specificity	Widely expressed.

Involvement in disease	Defects in CHUK are the cause of cocoon syndrome (COCOS) [MIM:613630]; also known as fetal encasement syndrome. COCOS is a lethal syndrome characterized by multiple fetal malformations including defective face and seemingly absent limbs, which are bound to the trunk and encased under the skin.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. I-kappa-B kinase subfamily. Contains 1 protein kinase domain.
Post-translational modifications	Phosphorylated by MAP3K14/NIK, AKT and to a lesser extent by MEKK1, and dephosphorylated by PP2A. Autophosphorylated. Acetylation of Thr-179 by Yersinia yopJ prevents phosphorylation and activation, thus blocking the I-kappa-B signaling pathway.
Cellular localization	Cytoplasm. Nucleus. Shuttles between the cytoplasm and the nucleus.

Images



Western blot - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

All lanes : Anti-IKK alpha antibody [Y463] (**ab32041**) at 1/1000 dilution (Purified)

Lane 1 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : Mouse kidney lysate
Lane 3 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 4 : Rat kidney lysate

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 85 kDa

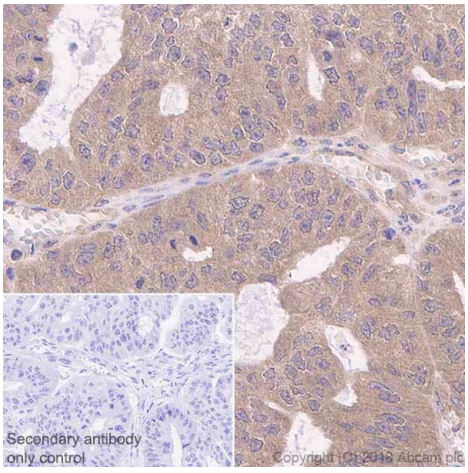
Western blot - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

Anti-IKK alpha antibody [Y463] (**ab32041**) at 1/10000 dilution + Daudi (Human Burkitt's lymphoma lymphoblast) whole cell lysate

Secondary

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/20000 dilution

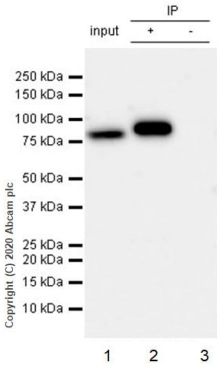
Predicted band size: 85 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

This data was developed using **ab32041**, the same antibody clone in a different buffer formulation.

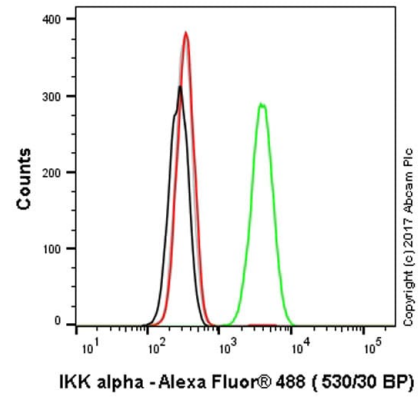
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian cancer tissue sections labeling IKK alpha with purified **ab32041** at 1/50 dilution (3.92 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunoprecipitation - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

Purified **ab32041** at 1/60 dilution (2µg) immunoprecipitating IKK alpha in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): **ab32041** + HeLa whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab169743 in HeLa whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 88 kDa
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

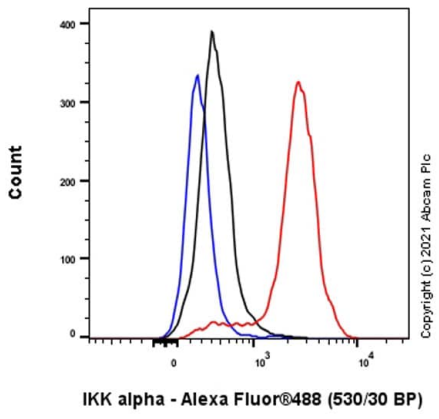
sodium azide (**ab32041**).



Flow Cytometry (Intracellular) - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

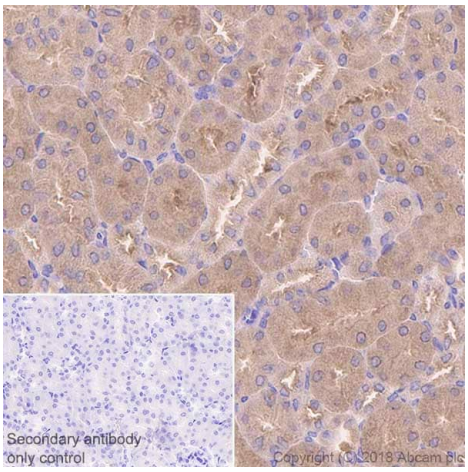
Overlay histogram showing HAP1 wildtype (green line) and HAP1-CHUK knockout cells (red line) stained with **ab32041**. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab32041**, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CHUK knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 80% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32041**).



Flow Cytometry (Intracellular) - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

This data was developed using **ab32041**, the same antibody clone in a different buffer formulation. Intracellular Flow Cytometry analysis of Daudi (Human Burkitt's lymphoma lymphoblast) cells labelling IKK alpha with purified **ab32041** 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). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling IKK alpha with purified **ab32041** at 1/50 dilution (3.92 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.

Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

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