abcam

ab169743

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

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	Y463
Purification technique	Affinity purification Protein A
Concentration	0.981 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Rat
Dilution info	-
Notes	**For unpurified use at 1/10000 - 1/50000.**

Species	Human
Dilution info	-
Notes	**For unpurified use at 1/10000 - 1/50000.**

Expected

Species	Mouse
Dilution info	-

Notes	**For unpurified use at 1/10000 - 1/50000.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	For unpurified use at 1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	-
Notes	For unpurified use at 1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Species	Rat
Dilution info	-
Notes	For unpurified use at 1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information CHUK

Function	Serine kinase that plays an essential role in the NF-kappa-B signaling pathway which is activated by multiple stimuli such as inflammatory cytokines, bacterial or viral products, DNA damages or other cellular stresses (PubMed:18626576, PubMed:9244310, PubMed:9252186, PubMed:9346484). Acts as a part of the canonical IKK complex in the conventional pathway of NF-kappa-B activation and phosphorylates inhibitors of NF-kappa-B on serine residues (PubMed:18626576, PubMed:35952808, PubMed:9244310, PubMed:9252186, PubMed:9346484). These modifications allow polyubiquitination of the inhibitors and subsequent degradation by the proteasome (PubMed:18626576, PubMed:9244310, PubMed:9252186, PubMed:9346484). In turn, free NF-kappa-B is translocated into the nucleus and activates the transcription of hundreds of genes involved in immune response, growth control, or protection against apoptosis (PubMed:18626576, PubMed:9244310, PubMed:9252186, PubMed:9346484). Negatively regulates the pathway by phosphorylating the scaffold protein TAXBP1 and thus promoting the assembly of the A20/TNFAIP3 ubiquitin-editing complex (composed of A20/TNFAIP3, TAX1BP1, and the E3 ligases ITCH and RNF11)

(PubMed:21765415). Therefore, CHUK plays a key role in the negative feedback of NF-kappa-B canonical signaling to limit inflammatory gene activation. 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 (PubMed:20501937). In turn, these complexes regulate genes encoding molecules involved in B-cell survival and lymphoid organogenesis. Also participates in the negative feedback of the non-canonical NF-kappa-B signaling pathway by phosphorylating and destabilizing MAP3K14/NIK. Within the nucleus, phosphorylates CREBBP and consequently increases both its transcriptional and histone acetyltransferase activities (PubMed:17434128). Modulates chromatin accessibility at NF-kappa-B-responsive promoters by phosphorylating histones H3 at 'Ser-10' that are subsequently acetylated at 'Lys-14' by CREBBP (PubMed:12789342). Additionally, phosphorylates the CREBBP-interacting protein NCOA3. Also phosphorylates FOXO3 and may regulate this pro-apoptotic transcription factor (PubMed:15084260). Phosphorylates RIPK1 at 'Ser-25' which represses its kinase activity and consequently prevents TNF-mediated RIPK1-dependent cell death (By similarity). Phosphorylates AMBRA1 following mitophagy induction, promoting AMBRA1 interaction with ATG8 family proteins and its mitophagic activity (PubMed:30217973).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab169743 is the carrier-free version of ab32041.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

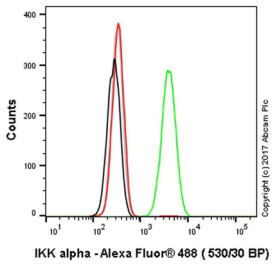
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

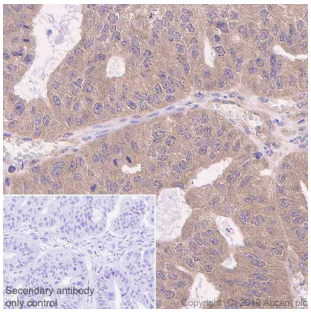
9 product images



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

IKK alpha Flow Cytometry (Intracellular) staining using rabbit Anti-IKK alpha antibody

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).

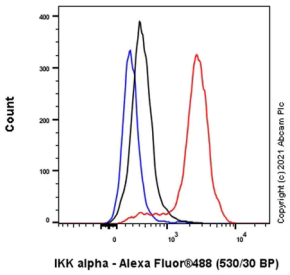


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

IKK alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IKK alpha antibody

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

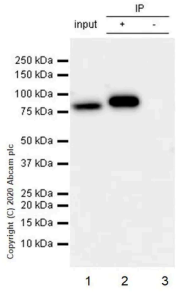
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian cancer tissue sections labeling IKK alpha with purified ab32041 at 1/50 dilution (3.92 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA 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.



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

IKK alpha Flow Cytometry (Intracellular) staining using rabbit Anti-IKK alpha antibody

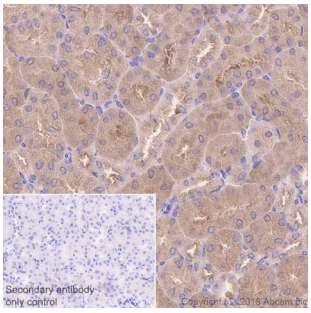
This data was developed using ab32041, the same antibody clone in a different buffer formulation. Intracellular Flow Cytometry analysis of Daudi (Human Burkitt's lymphoma lymphoblast) cells labelling IKK alpha with purified ab32041 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde 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).



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).
All lanes:
Immunoprecipitation - Anti-IKK alpha antibody [Y463] (ab32041)

Predicted band size: 84 kDa

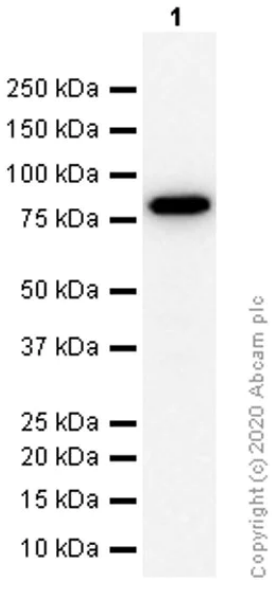


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

IKK alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IKK alpha antibody

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

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.



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

IKK alpha Western blot staining of Daudi (Human Burkitt's lymphoma lymphoblast) whole cell lysate using rabbit Anti-IKK alpha antibody

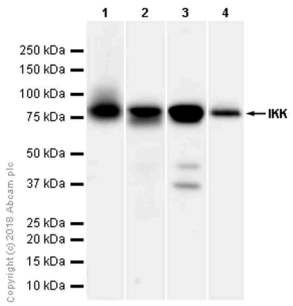
All lanes:
Western blot - Anti-IKK alpha antibody [Y463] (ab32041) at 1/10000 dilution

All lanes:
Daudi (Human Burkitt's lymphoma lymphoblast) whole cell lysate

Secondary

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

Predicted band size: 84 kDa



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

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

Lane 1:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 15 µg

Lane 2:
Mouse kidney lysate at 15 µg

Lane 3:
C6 (Rat glial tumor glial cell) whole cell lysate at 15 µg

Lane 4:
Rat kidney lysate at 15 µg

Secondary

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

Predicted band size: 84 kDa

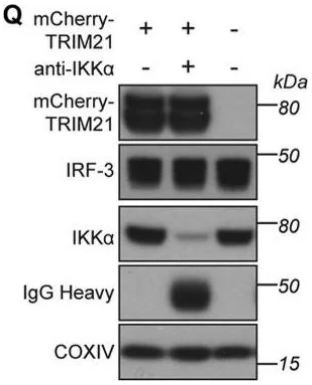


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Western blot - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

IKK alpha Western blot staining using rabbit Anti-IKK alpha antibody

Western Blotting using Recombinant Anti-IKK alpha antibody [Y463] - BSA and Azide free, ab169743. Publication image from Clift, D. et al., 2017, Cell, 29153837. Legend direct from paper.

TRIM21 Overexpression Does Not Perturb Cells, Related to Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7(A) Oocytes microinjected with either water (control) or Trim21 mRNA (TRIM21 OE) were imaged for sixteen hours following release from prophase arrest. Microtubules and chromosomes were labeled with mEGFP-Map4 and H2B-mCherry respectively. Time shows hours and minutes (h:min) from nuclear envelope breakdown (NEBD). Scale bars, 10 µm. White dashed line outlines oocyte. Yellow dashed line outlines nucleus.(B–G) Key events in meiosis were quantified. Number of oocytes is specified in brackets. P values were calculated with Fisher’ s exact test (B, D, E and G) or Student’ s t test (C and F).(H and I) NIH 3T3, NIH 3T3-mCherry-mTRIM21, HEK293T and HEK293T-mCherry-hTRIM21 cell lines were analyzed by flow cytometry. At least 2000 cells were counted for each condition. Percentages correspond to mCherry-positive cells falling within the gate drawn.(J) NIH 3T3 and NIH 3T3-mCherry-mTRIM21 cells (NIH 3T3 mix) or HEK293T and HEK293T-mCherry-hTRIM21 cells (HEK293T mix) were mixed 50:50 and percentage mCherry cells analyzed by flow cytometry every 24h for 7 days. At least 10,000 cells counted at each time point. Error bars show s.d. Data from 3 independent replicates.(K–M) Transcriptomes of NIH 3T3-mCherry-mTRIM21 cells were compared to wild-type NIH 3T3 cells by RNA-seq analysis (STAR Methods) following electroporation with PBS (K), BSA (L) or IgG (M). A total of only 8 protein-encoding transcripts were consistently downregulated more than 2-fold in the NIH 3T3-mCherry-mTRIM21 cells (Kdm5d, Ddx3y, Eif2s3y, Uty, Asb4, Fat4, Papss2 and Lama2), although we cannot rule out the possibility that this is an indirect consequence of lentivirus construct integration rather than a direct consequence of TRIM21 overexpression.(N–P) Relative expression levels of genes encoding proteins reported to be ligands of TRIM21. Taken from RNA sequencing data. Irf8 was not detected.See also Tables S1, S2, and S3.(Q) HEK293T and HEK293T-mCherry-hTRIM21 cells were electroporated with PBS or anti-IKKα antibody and whole cell lysates harvested 3 hours later for immunoblotting. IRF-3 protein levels are unaffected by TRIM21 overexpression or activation.

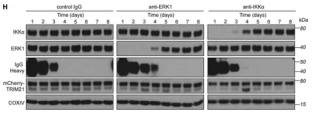


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Western blot - Anti-IKK alpha antibody [Y463] - BSA and Azide free (ab169743)

IKK alpha Western blot staining using rabbit Anti-IKK alpha antibody

Western Blotting using Recombinant Anti-IKK alpha antibody [Y463] - BSA and Azide free, ab169743. Publication image from Clift, D. et al., 2017, Cell, 29153837. Legend direct from paper.

Time Frame for Protein Depletion by Trim-Away, Related to Figure 5(A–F) NIH 3T3-mCherry-TRIM21 cells were taken up into the Neon Pipette Tip and either electroporated or not (mock). Percentage of dead cells was determined using the trypan blue exclusion assay (A). For long-term analysis of cellular behavior cells were imaged every 15 min for 70 h following electroporation (B-F). For the growth curves cell density was normalized to the initial density once cells had adhered (D). For adherence analysis the percentage of spread cells was quantified in each frame (B and C). Doubling times (E and F) were calculated taking the 5 h time point as reference, because cells were fully adhered then. Data from three independent experiments. Error bars show s.d. P values were calculated with Student’ s t test. See also Movie S4.(G and H) HEK293T-mCherry-TRIM21 cells were electroporated with either control IgG, anti-ERK1 or anti-IKKα antibodies and whole cell lysates harvested at the indicated times after electroporation for immunoblotting.(I–M) NIH 3T3-mCherry-TRIM21 cells were electroporated with control IgG or anti-Pericentrin antibody (BD611815) and 3 hours later analyzed for Pericentrin (I and K) or Cdk5rap2 (J and L) localization or cell lysates were immunoblotted for the indicated proteins (M). Merge also shows DNA stained with Hoechst. Number of cells in brackets. Scale bars, 5 µm. Data from two independent experiments. P values were calculated with Fisher’ s exact (K) or Student’ s t test (L).

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