Product Datasheet

IkB-alpha Antibody (6A920) - BSA Free
NB100-56507

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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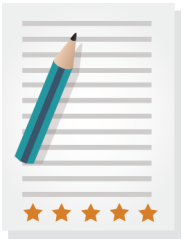
Updated 4/13/2025 v.20.1



NB100-56507

IkB-alpha Antibody (6A920) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	6A920
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description

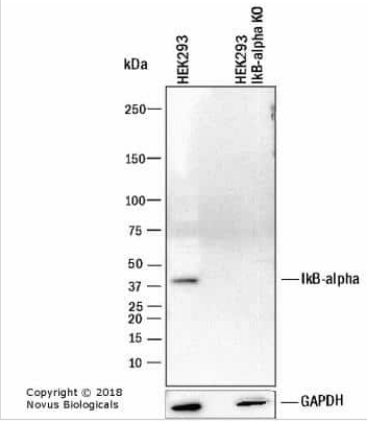
Host	Mouse
Gene ID	4792
Gene Symbol	NFKBIA
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in the scientific literature (PMID: 23840265).
Immunogen	Partial recombinant protein corresponding to amino acid residues 32-291 of human IkB-alpha.

Product Application Details

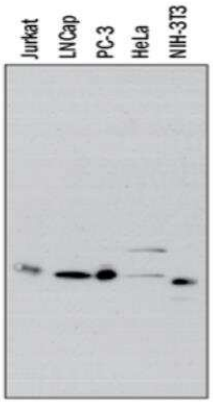
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, CyTOF-ready, Knockout Validated
Recommended Dilutions	Western Blot 1-2 ug/ml, Simple Western 1:20, Flow Cytometry 0.25-1 ug/10^6 cells, Immunohistochemistry 1:20-1:1000, Immunocytochemistry/ Immunofluorescence 1:100-1:1000, Immunoprecipitation 1 ug/ml, Immunohistochemistry-Paraffin 1:100, CyTOF-ready, Knockout Validated
Application Notes	A 40 kDa band is observed.

Images

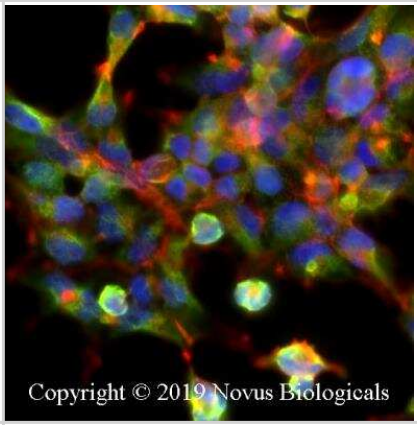
Western Blot: IkB-alpha Antibody (6A920) [NB100-56507] - Western blot shows lysates of HEK293 human embryonic kidney parental cell line and IkB-alpha knockout (KO) HEK293 human embryonic kidney cell line. PVDF membrane was probed with 2 ug/ml of Mouse Anti-Human IkB-alpha monoclonal Antibody (Catalog # NB100-56507) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody. Specific band was detected for IkB-alpha at approximately 38 kDa (as indicated) in the parental HEK293 celll line, but is not detectable in the knockout HEK293 cell line. This experiment was conducted under reducing conditions.



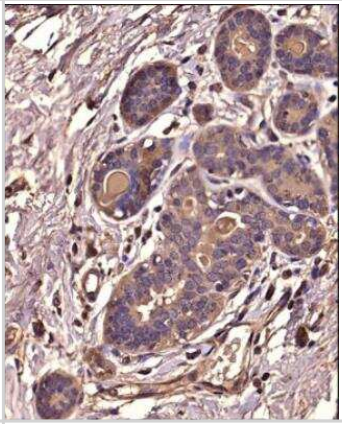
Western Blot: IkB-alpha Antibody (6A920) [NB100-56507] - Lysates of Jurkat human acute T cell leukemia cell line, LNCaP human prostate cancer cell line, PCx2011;3 human prostate cancer cell line, HeLa human cervical epithelial carcinoma cell line, and NIHx2011;3T3 mouse embryonic fibroblast cell line. PVDF membrane was probed with 0.5 ug/mL mouse anti-IkB-a monoclonal (NB100-56507, Novus Biologicals), followed by 1:2000 dilution of the appropriate HRP-conjugated secondary antibody, donkey anti-mouse IgG.



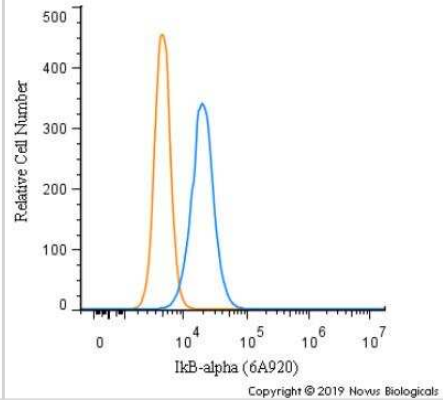
Immunocytochemistry/Immunofluorescence: IkB-alpha Antibody (6A920) [NB100-56507] - Hek293 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-IkB-alpha (6A920) at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

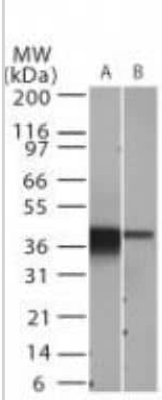
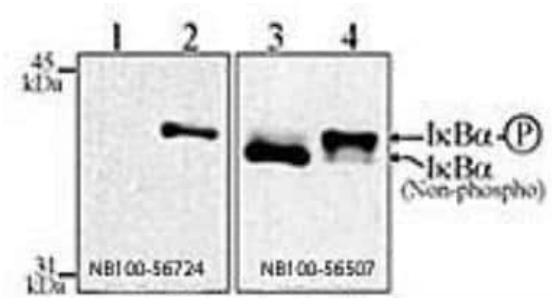
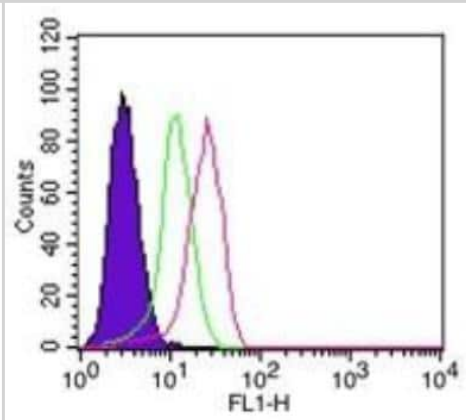
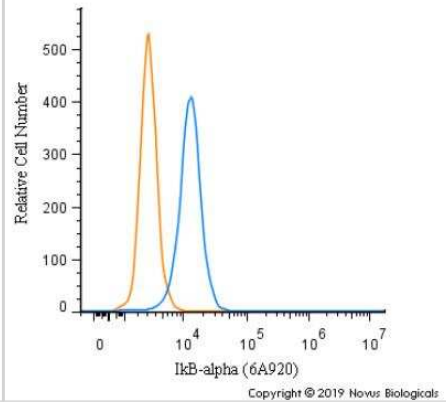


Immunohistochemistry-Paraffin: IkB-alpha Antibody (6A920) [NB100-56507] - Analysis of a FFPE tissue section of human breast using 1:200 dilution of IkB-alpha clone 6A920 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. Cytoplasmic and membrane staining of glandular cells was observed.

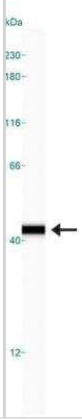


Flow Cytometry: IkB-alpha Antibody (6A920) [NB100-56507] - An intracellular stain was performed on NIH3T3 cells with IkB-alpha Antibody [6A920] NB100-56507 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550.



Western Blot: IkB-alpha Antibody (6A920) [NB100-56507] - IkBa using NB100-56507 at 2 ug/ml in (A) Daudi and (B) NIH 3T3 whole cell lysate.	
Western Blot: IkB-alpha Antibody (6A920) [NB100-56507] - Jurkat cells were treated for 30 min with 100 ug/ml ALLN (N-Acetyl-Leu-Leu-Norleucinal; a Calpain inhibitor and also proteasome inhibitor that prevents IkBa dephosphorylation) followed by incubation with (lanes 2 & 4) or without 1 nM TNF-a (1 & 3). The membranes were blotted with NB100-56724 (lanes 1 & 2) or NB100-56507 (that recognizes both non-phospho and phosphorylated forms of IkBa) and immunoreactivity was detected by ECL. The data shows that NB100-56724 detects specifically the phosphorylated form of IkBa.	
Flow Cytometry: IkB-alpha Antibody (6A920) [NB100-56507] - Intracellular staining of 10^6 ThP-1 cells using 0.25 ug of NB100-56507. Shaded histogram represents cells alone, green represents the isotype control, and red represents the IkBa antibody. Novus's intracellular flow kit was used for this test, and an anti-mouse IgG FITC conjugated secondary.	
Flow Cytometry: IkB-alpha Antibody (6A920) [NB100-56507] - An intracellular stain was performed on Hek293 cells with IkB-alpha Antibody [6A920] NB100-56507 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550.	

Simple Western: IkB-alpha Antibody (6A920) [NB100-56507] - Simple Western lane view shows a specific band for IKB alpha in 0.5 mg/ml of NIH-3T3 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Publications

Lan Zhao, Yumei Lai, Hongli Jiao, Jian Huang Nerve growth factor receptor limits inflammation to promote remodeling and repair of osteoarthritic joints Nature Communications 2024-04-15 [PMID: 38622181]

Sartori G, Napoli S, Cascione L et al. ASB2 is a direct target of FLI1 that sustains NF-?B pathway activation in germinal center-derived diffuse large B-cell lymphoma Journal of Experimental & Clinical Cancer Research 2021-11-11 [PMID: 34763718] (Western Blot)

Zeng L, Herdman DS, Lee SM et al. Loss of cAMP signaling in CD11c immune cells protects against diet-induced obesity Diabetes 2023-05-31 [PMID: 37257047] (WB)

S Ohta, M Asanoma, N Irie, N Tachibana, M Kohno Soy Phospholipids Exert a Renoprotective Effect by Inhibiting the Nuclear Factor Kappa B Pathway in Macrophages Metabolites, 2022-04-06;12(4):. 2022-04-06 [PMID: 35448517] (Simple Western)

Stanfield BA, Purves T, Palmer S, et al. IL-10 and class 1 histone deacetylases act synergistically and independently on the secretion of proinflammatory mediators in alveolar macrophages PloS one 2021-01-20 [PMID: 33471802] (WB, Mouse)

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

Details:
Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Kamohara A, Hirata H et al. IgG immune complexes with Staphylococcus aureus protein A enhance osteoclast differentiation and bone resorption by stimulating Fc receptors and TLR2. Int Immunol 2019-12-11 [PMID: 31713625] (WB, Mouse)

Korgaonkar AA, Nguyen S, Li Y et al. Distinct cellular mediators drive the Janus faces of toll-like receptor 4 regulation of network excitability which impacts working memory performance after brain injury Brain Behav. Immun. 2020-04-04 [PMID: 32259563] (WB, Rat)

Chen Z, Yao L, Liu Y et al. Astragaloside IV regulates NF-kappa B mediated cellular senescence and apoptosis of hepatic stellate cells to suppress PDGF BB induced activation Exp Ther Med 2019-09-25 [PMID: 31641375] (WB, Rat)

Zhu F, Willette-Brown J, Song NY et al. Autoreactive T Cells and Chronic Fungal Infection Drive Esophageal Carcinogenesis. Cell Host Microbe. 2017-04-12 [PMID: 28407484]

Tomasi ML, Ramani K, Ryoo M. Ubiquitin-Conjugating Enzyme 9 Phosphorylation as a Novel Mechanism for Potentiation of the Inflammatory Response. Am. J. Pathol. 2016-09-01 [PMID: 27561301] (WB)

Gao Q, Liu Y, Wu Y et al. IL-17 intensifies IFN-gamma-induced NOS2 upregulation in RAW 264.7 cells by further activating STAT1 and NF-kB Int. J. Mol. Med. 2015-12-11 [PMID: 26677135] (WB, Mouse)

More publications at http://www.novusbio.com/NB100-56507

Procedures

Western Blot Protocol for IkB-alpha Antibody (NB100-56507)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers inst

Immunohistochemistry-Paraffin Protocol for IkB-alpha Antibody (NB100-56507)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Flow (Intracellular) Protocol for IkB-alpha Antibody (NB100-56507)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunocytochemistry/Immunofluorescence Protocol for IkB-alpha Antibody (NB100-56507)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

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Products Related to NB100-56507

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)
NBP2-24815	IkB-alpha Antibody (6A920) [Biotin]

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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