Product Datasheet

IKK beta Antibody (10AG2) - BSA Free
NB100-56509SS

Unit Size: 0.025 mg

Store at -20C. Avoid freeze-thaw cycles.

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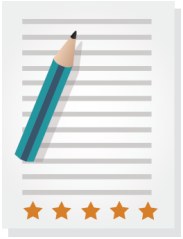
Updated 4/13/2025 v.20.1



NB100-56509SS

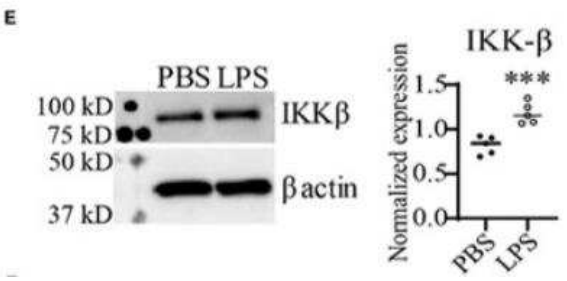
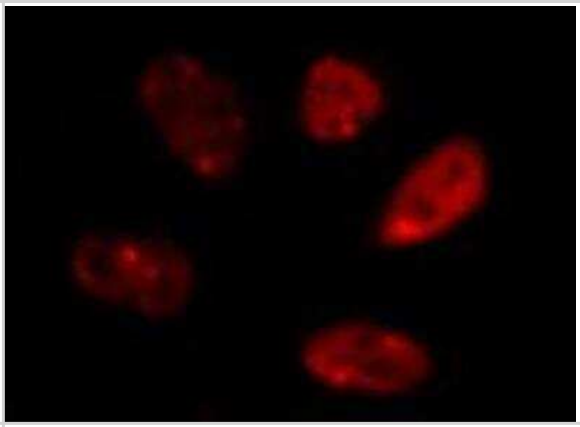
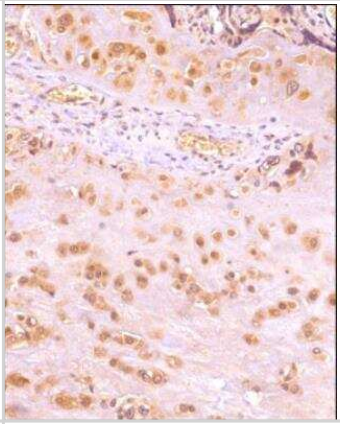
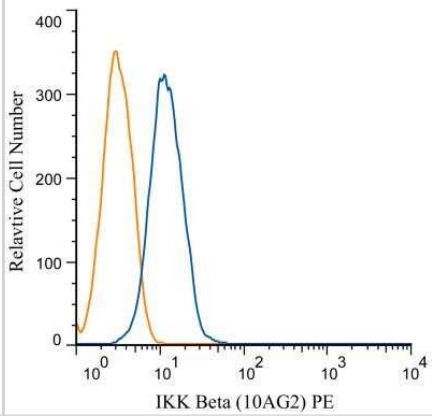
IKK beta Antibody (10AG2) - BSA Free

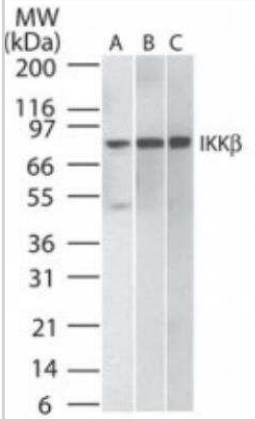
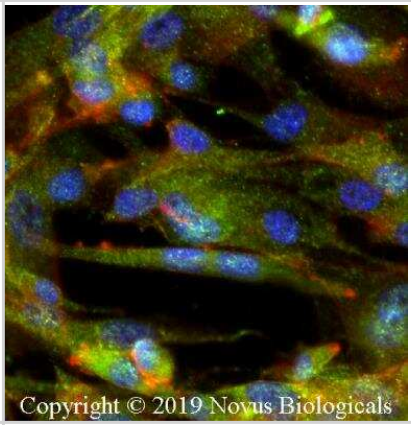
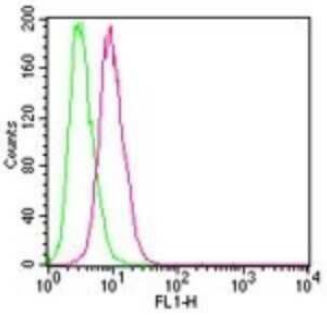
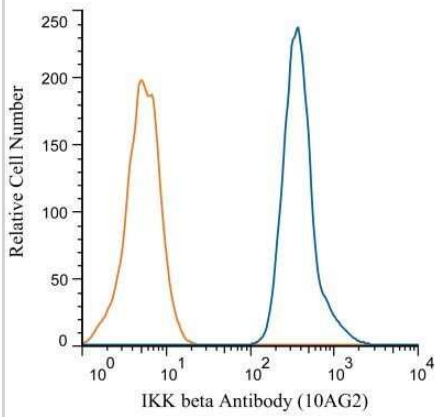
Product Information	
Unit Size	0.025 mg
Concentration	1.0 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	10AG2
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	87 kDa

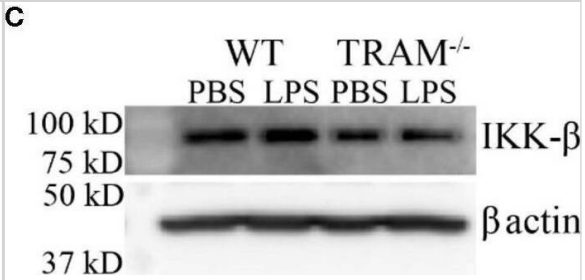
Product Description	
Host	Mouse
Gene ID	3551
Gene Symbol	IKBKB
Species	Human, Mouse, Rat
Reactivity Notes	Use in Rat reported in scientific literature (PMID:34333329)
Immunogen	Full-length recombinant human IKK beta protein (NP_001547).

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, CyTOF-ready
Recommended Dilutions	Western Blot 2 - 4 ug/ml, Simple Western 1:12.5, Flow Cytometry 0.25 - 0.5 ug/ 10^6 cells, Immunohistochemistry 1:10 - 1:500, Immunocytochemistry/ Immunofluorescence 2-5 ug/ml. Use reported in scientific literature (PMID 24825920), Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:10 - 1:500. Use reported in scientific literature (Page et al), Immunoblotting reported in scientific literature (PMID 20103608), CyTOF-ready
Application Notes	In Simple Western only 10 - 15 ul of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 1:12.5, apparent MW was 90 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors. This antibody is CyTOF ready.

Images

Western Blot: IKK beta Antibody (10AG2) [NB100-56509] - Representative western blot of IKKB (E) in monocytes with quantification on the right. All data are representative of 5 separate experiments. Unpaired student t-test. ***p < 0.001. Image collected and cropped by CiteAb from the following publication (doi.org/10.3389/fimmu.2020.01478) licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: IKK beta Antibody (10AG2) [NB100-56509] - Analysis of IKK beta Antibody in HeLa cells. Antibody dilution 5 ug/ml. Image from verified customer review.	
Immunohistochemistry-Paraffin: IKK beta Antibody (10AG2) [NB100-56509] - Analysis of a FFPE tissue section of human placenta using 1:200 dilution of IKK beta clone 10AG2 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.	
Flow Cytometry: IKK beta Antibody (10AG2) [NB100-56509] - Analysis of PE conjugate of NB100-56509. An intracellular stain was performed on Jurkat cells with IKK Beta antibody (10AG2) NB100-56509PE (blue) and a matched isotype control NBP2-27287AF488 (orange). Cells were fixed with 4% PFA and then permeablized wi	

Western Blot: IKK beta Antibody (10AG2) [NB100-56509] - Analysis of A) human Daudi, B) HeLa, and C) mouse NIH3T3 lysate probed with IKKbeta antibody at 2 ug/ml.	
Immunocytochemistry/Immunofluorescence: IKK beta Antibody (10AG2) [NB100-56509] - U-87 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-IKK beta Antibody (10AG2) 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 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Flow Cytometry: IKK beta Antibody (10AG2) [NB100-56509] - Analysis using the Alexa Fluor (R) 488 conjugate of NB100-56509. Staining of IKKbeta in HEK 293 cells using 0.1 ug of Alexa Fluor 488-conjugated antibody. Green histogram represents the isotype control , red represents the IKKbeta antibody.	
Flow Cytometry: IKK beta Antibody (10AG2) [NB100-56509] - Analysis using Alexa Fluor (R) 647 conjugate of NB100-56509. An intracellular stain was performed on HeLa cells with IKK beta antibody (10AG2) NB100-56509 (blue) and a matched isotype control NBP2-27287 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. 1 ug of antibody was added to 100 uL of staining buffer and cells were incubated for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	

Simple Western: IKK beta Antibody (10AG2) [NB100-56509] - Lane view shows a specific band for IKK beta in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: IKK beta Antibody (10AG2) - BSA Free [NB100-56509] - TRAM is required for the pro-inflammatory polarization of macrophages by SLD-LPS. (A,B) Flow cytometry analysis of Ly6C (A) or CD200R (B) expression in WT or TRAM−/− live-cell-gated macrophages with geometric MFI quantification on right. Pink = PBS, Blue = 100 pg/ml LPS. (C–E) Representative Western blots of IKK-β (C), p-p65 (D), & total NF-κB p65 (E) expression in WT & TRAM−/− monocytes treated with either PBS or 100 pg/mL LPS for 5 days. Quantification is depicted on the right. (F) Ratio of p-p65/total p65 (p-p65 levels from (D) divided by corresponding total p65 levels). All data are representative of at least 3 separate experiments (n = 3) for (A,B) & n = 6 for (C–F). ANOVA with Tukey's multiple comparisons test (A,B); unpaired student t-test (C–F) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, n.s, non-significant; MFI, Mean fluorescence intensity. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32765513), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

McCorkell KA, Jayachandran N, Cully MD Et al. Lymph node formation and B cell homeostasis require IKK-alpha in distinct endothelial cell-derived compartments Proceedings of the National Academy of Sciences of the United States of America 2021-11-30 [PMID: 34810256]

Yan Huo, Abudureheman Mijiti, Ruonan Cai, Zhaohua Gao, Maierpu Aini, Abudukadier Mijiti, Zhaoling Wang, Rui Qie Scutellarin alleviates type 2 diabetes (HFD/low dose STZ)-induced cardiac injury through modulation of oxidative stress, inflammation, apoptosis and fibrosis in mice. Human & experimental toxicology 2022-03-07 [PMID: 34610774]

Borar P, Biswas T, Huxford T et al. Dual-specific autophosphorylation of kinase IKK2 enables phosphorylation of substrate I?B? without requiring ATP bioRxiv 2023-06-27 (Western Blot, Mouse)

Rahtes, A & Li, L. Polarization of Low-Grade Inflammatory Monocytes Through TRAM-Mediated Up-Regulation of Keap1 by Super-Low Dose Endotoxin. Front Immunol 2020-08-09 [PMID: 32765513] (IF/IHC, Human)

Karim K, Giribabu N, Salleh N Marantodes pumilum Var Alata (Kacip Fatimah) ameliorates derangement in RANK/RANKL/OPG pathway and reduces inflammation and oxidative stress in the bone of estrogen-deficient female rats with type-2 diabetes Phytomedicine : international journal of phytotherapy and phytopharmacology 2021-07-18 [PMID: 34333329] (IF/IHC)

Lim Y, Ooi K, Subramaniam M, et al. Apoptotic and cytostatic actions of maslinic acid in colorectal cancer cells through possible IKK-β inhibition Asian Pacific Journal of Tropical Biomedicine 2021-01-21

Best KT, Nichols AEC, Knapp E et al. NF-kappaB activation persists into the remodeling phase of tendon healing and promotes myofibroblast survival Nat Commun 2020-11-13 [PMID: 33203721] (WB, Human, Mouse)

Reid M A, Lowman X H et al. IKK bet promotes metabolic adaptation to glutamine deprivation via phosphorylation and inhibition of PFKFB3. Genes Dev 2016-08-15 [PMID: 27585591] (WB, Human)

De S, Karim F, Kiessu E et al. Mechanism of dysfunction of human variants of the IRAK4 kinase and a role for its kinase activity in interleukin-1 receptor signaling J. Biol. Chem. 2018-08-16 [PMID: 30115681] (WB, Human)

Abdi K, Lai CH, Paez-Gonzalez P et al. Uncovering inherent cellular plasticity of multiciliated ependyma leading to ventricular wall transformation and hydrocephalus. Nat Commun 2018-04-25 [PMID: 29695808] (Mouse)

Cleary MM, Mansoor A, Settelmeyer T et al. NFkB signaling in alveolar rhabdomyosarcoma Dis Model Mech 2017-09-01 [PMID: 28883017] (WB, Mouse)

Kirkley KS, Walton KD, Duncan C, Tjalkens RB. Spontaneous Development of Cutaneous Squamous Cell Carcinoma in Mice with Cell-specific Deletion of Inhibitor of kB Kinase 2 Comp. Med. 2017-10-01 [PMID: 28935002] (IHC-P, Mouse)

More publications at http://www.novusbio.com/NB100-56509

Procedures

Western Blot Protocol for IKK beta Antibody (NB100-56509)
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 instructi

Immunohistochemistry-Paraffin Protocol for IKK beta Antibody (NB100-56509)
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.

Immunocytochemistry/Immunofluorescence Protocol for IKK beta Antibody (NB100-56509)
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.

Flow (Intracellular) Protocol for IKK beta Antibody (NB100-56509)

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.

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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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