# Product Datasheet

## IKK alpha Antibody (14A231) - BSA Free
## NB100-56704

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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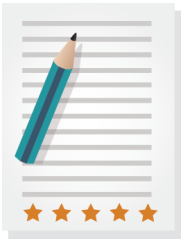
Updated 4/13/2025 v.20.1





# NB100-56704

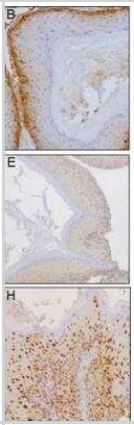
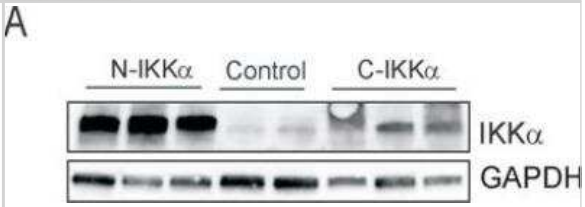
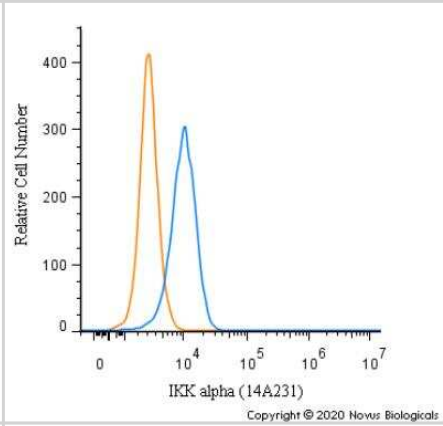
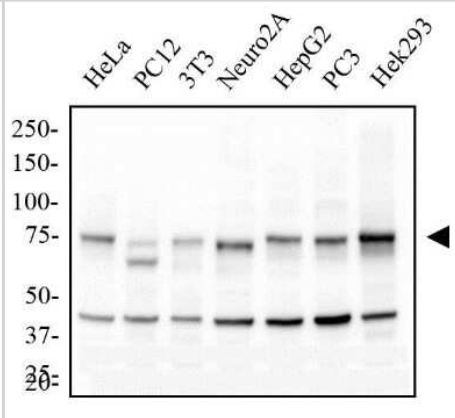
IKK alpha Antibody (14A231) - BSA Free

| Product Information | |
|---|---|
| **Unit Size** | 0.1 mg |
| **Concentration** | 1 mg/ml |
| **Storage** | Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles. |
| **Clonality** | Monoclonal |
| **Clone** | 14A231 |
| **Preservative** | 0.05% Sodium Azide |
| **Isotype** | IgG1 Kappa |
| **Purity** | Protein G purified |
| **Buffer** | PBS |

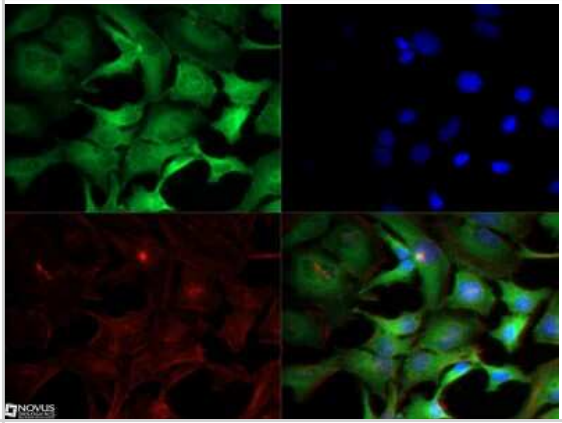
| Product Description | |
|---|---|
| **Host** | Mouse |
| **Gene ID** | 1147 |
| **Gene Symbol** | CHUK |
| **Species** | Human, Mouse, Rat, Primate |
| **Reactivity Notes** | New World Monkey. Use in Mouse reported in scientific literature (PMID:35121655). |
| **Immunogen** | This antibody was raised against a His-tagged full-length human IKK alpha protein. |

| Product Application Details | |
|---|---|
| **Applications** | Western Blot, Simple Western, Chromatin Immunoprecipitation, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP) |
| **Recommended Dilutions** | Western Blot 1 ug/ml, Simple Western 10 ug/ml, Chromatin Immunoprecipitation 1:10 - 1:500. Use reported in scientific literature (PMID 27705798), Flow Cytometry (Intracellular): 0.25-0.5 ug/10^6 cells, Immunohistochemistry 1:200. Use reported in scientific literature (PMID 27121058), Immunocytochemistry/ Immunofluorescence 1:10, Immunoprecipitation 1-2 ug/ml, Immunohistochemistry-Paraffin 5ug/ml, Immunohistochemistry-Frozen reported in scientific literature (PMID 25133425), Flow (Intracellular) 0.25-0.5 ug/10^6 cells. Use reported in scientific literature (PMID 24804954), Chromatin Immunoprecipitation (ChIP) 1:10-1:500 |
| **Application Notes** | In Western blot a 85 kDa band is observed. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.<br>See Simple Western Antibody Database for Simple Western validation: Tested in Daudi and U937 lysate 0.5 mg/mL, separated by Size, antibody dilution of 10 ug/mL. Separated by Size-Wes, Sally Sue/Peggy Sue. |

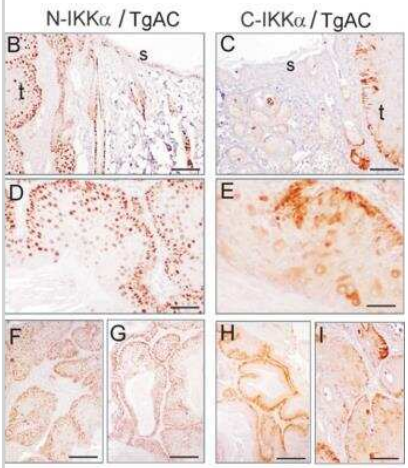
## Images

| | |
|---|---|
| Immunohistochemistry-Paraffin: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - Analysis of human IKK alpha in tumors of the three groups B (C-IKK alpha), E (Control), H (N-IKK alpha). Specific staining of human IKK alpha-using the NB100-56704 antibody. Scale bar: 70um. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/article/8792/text/) licensed under a CC-BY license. |   |
| Western Blot: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - Western blot showing the increased expression of IKK-alpha in transgenic mice. Image collected and cropped by CiteAb from the following publication (oncotarget.com/fulltext/8792), licensed under a CC-BY license. |   |
| Flow Cytometry: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - An intracellular stain was performed on Hek293 cells with IKK alpha Antibody (14A231) NB100-56704 (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 2.5 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher). |   |
| Western Blot: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - IKK alpha Antibody (14A231) [NB100-56704] - Total protein from various Human, Mouse and Rat cell lines were separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/mL anti-IKK-alpha in 1% non-fat milk in TBST and detected with an anti-mouse HRP secondary antibody using chemiluminescence. |   |

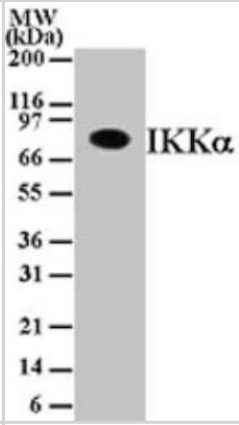
Immunocytochemistry/Immunofluorescence: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - Antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red). An antibody dilution of 1:10 was used. Image objective 40X.



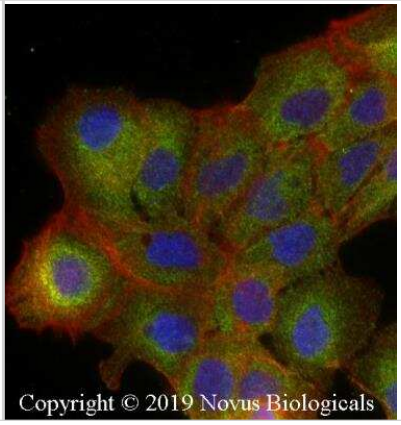
Immunohistochemistry: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - B-I. Immunohistochemistry showing the expression of the transgenic protein in N-IKKa and C-IKKa tumors. Staining with NB100-56704 antibody is shown. (B, C) Representative images showing the expression of transgenic IKKa in tumors and adjacent skin of N-IKKa/TgAC mice (B), and C-IKKa/TgAC animals (C). (D, E) Detail showing the nuclear (D) or cytoplasmic (E) localization of the transgenic IKKa in tumors. (F, G) Similar levels of expression of the transgenic IKKa in different N-IKKa tumors. By contrast variable levels of expression of the transgene are observed between different C-IKKa tumors (H, I). t: tumor; s: non-tumoral skin. Scale bar: (B, C) 100um; (D, E) 80 um; (F-I) 200 um. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/article/8792/text/) licensed under a CC-BY license.

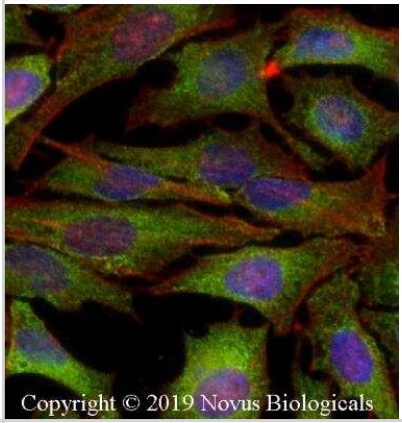
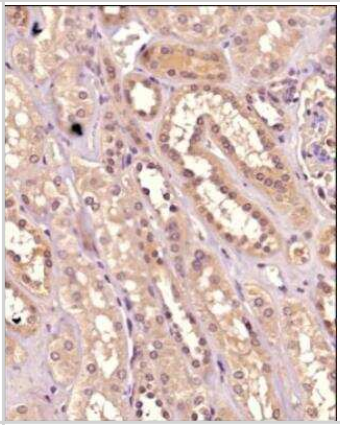
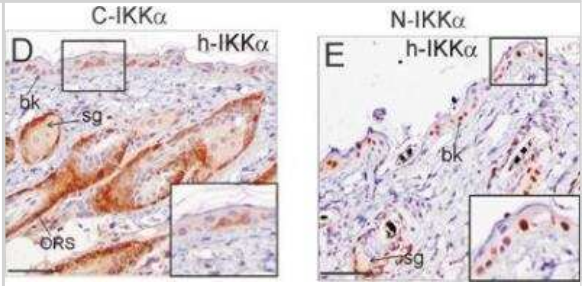



Western Blot: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - IKK alpha Antibody (14A231) [NB100-56704] - Analysis of IKK alpha in Daudi cell lysate using IKK alpha antibody at 1 ug/mL.

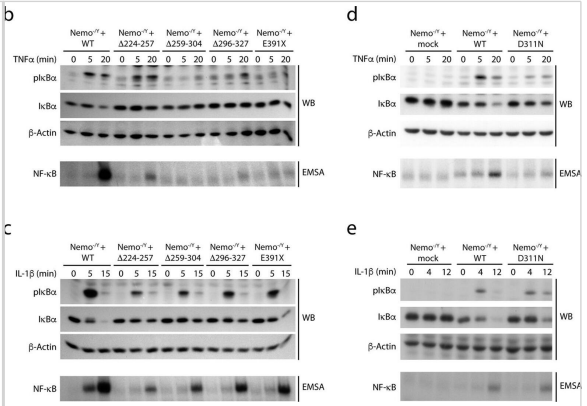



Immunocytochemistry/Immunofluorescence: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-IKK alpha Antibody (14A231) at 2 ug/mL overnight 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.

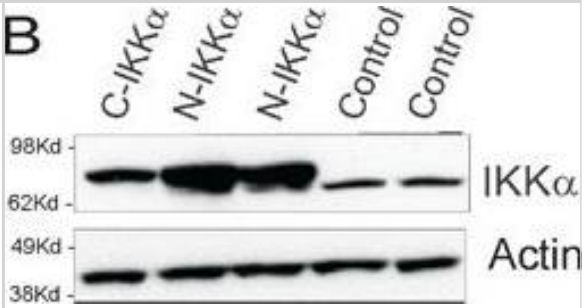


| | |
|---|---|
| Immunocytochemistry/Immunofluorescence: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-IKK alpha Antibody (14A231) 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: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - Analysis of a FFPE tissue section of human kidney using 1:200 dilution of IKK alpha clone 14A231 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. |  |
| Immunohistochemistry-Paraffin: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - Expression of the transgenic IKK alpha protein in skin of C-IKK alpha and N-IKK alpha mice. D-E. Expression of exogenous IKK alpha protein in back skin of 1-month-old mice. Immunostaining with the NB100-56704 anti-IKK alpha antibody is shown. Note the cytoplasmic expression of the transgene in the C-IKK alpha mice (D). By contrast, it is located in the nuclei of cells in the N-IKK alpha mice (E). In both types of transgenic mice the exogenous IKK alpha is expressed in basal keratinocytes (bk), in the outer root sheath of hair follicles (ORS) and in cells surrounding the sebaceous glands (sb). Scale bar: 60um. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/article/8792/text/) licensed under a CC-BY license. |  |
| Simple Western: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - IKK alpha Antibody (14A231) [NB100-56704] - Image shows a specific band for IKK alpha in 0.5 mg/mL of Daudi (left) and U937 (right) lysate. This exeriment was performed under reducing conditions using the 12-230 kDa separation system. |  |

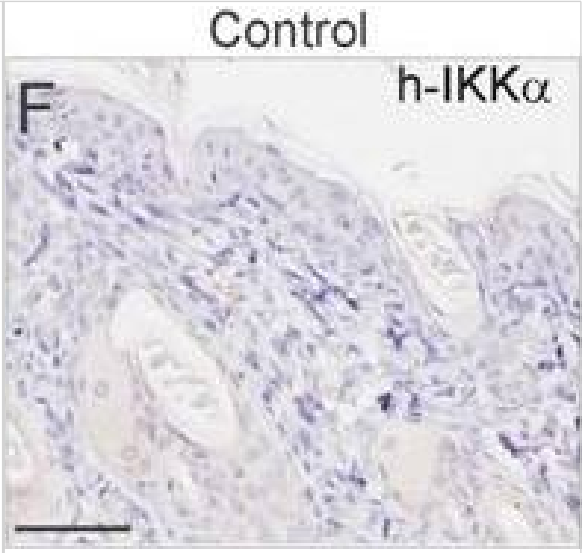
Western Blot: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - NEMO-Ub binding is essential for TNFα signaling, but not for IL-1β signaling.(a) Schematic illustration of NEMO deletions or point mutations. (b,c) Effects of NEMO C-terminal deletion mutants on TNFα & IL-1β signaling. NEMO−/Y MEFs were reconstituted with mock, NEMO WT or different C-terminal deletion constructs & stimulated with TNFα (b) or IL-1β (c). Effects on NF-κB signaling were investigated by determining IκBα phosphorylation & degradation in Western Blots as well as NF-κB-DNA binding by EMSA. (d,e) Effects of NEMO D311N on TNFα & IL-1β signaling. NEMO−/Y MEFs were reconstituted with mock, NEMO WT or NEMO D311N point mutant & stimulated with TNFα (d) or IL-1β (e). Analysis on NF-κB signaling were performed as in (b,c). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26740240), licensed under a CC-BY license. Not internally tested by Novus Biologicals.




Western Blot: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - Expression of the transgenic IKKα protein in skin of C-IKKα & N-IKKα miceA. Recombinant DNA constructs employed to generate both transgenic mice lines. For C-IKKα mice generation, the nuclear localization signal (NLS) was removed from the sequence of the human IKKα cDNA employed. In the construct used for generation of the N-IKKα mice an extra NLS signal was added. WT IKKα; wild type IKKα. B. Western blot of total protein extracts showing IKKα expression in back skin of Control & C-and N-IKKα mice. Actin was used as a loading control. C. Representative example of the K5 staining in back skin section of Control mice. D-E. Expression of exogenous IKKα protein in back skin of 1-month-old mice. Immunostaining with the NB100-56704 anti-IKKα antibody is showed; similar results were obtained with the H00001147-M04 IKKα antibody (not shown). Note the cytoplasmic expression of the transgene in the C-IKKα mice (D). By contrast, it is located in the nuclei of cells in the N-IKKα mice (E). In both types of transgenic mice the exogenous IKKα is expressed in basal keratinocytes (bk), in the outer root sheath of hair follicles (ORS) & in cells surrounding the sebaceous glands (sb). F. Back skin section of Control mice. The NB100-56704 antibody used does not recognize the endogenous IKKα in immunohistochemical assays. G. Endogenous IKKα expression in control mice using the IKKα (sc-7182) antibody. Scale bar: (C) 70 μm; (D-G) 60 μm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27121058), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemistry-Paraffin: IKK alpha Antibody (14A231) - BSA Free [NB100-56704] - Expression of the transgenic IKKα protein in skin of C-IKKα & N-IKKα miceA. Recombinant DNA constructs employed to generate both transgenic mice lines. For C-IKKα mice generation, the nuclear localization signal (NLS) was removed from the sequence of the human IKKα cDNA employed. In the construct used for generation of the N-IKKα mice an extra NLS signal was added. WT IKKα; wild type IKKα. B. Western blot of total protein extracts showing IKKα expression in back skin of Control & C-and N-IKKα mice. Actin was used as a loading control. C. Representative example of the K5 staining in back skin section of Control mice. D-E. Expression of exogenous IKKα protein in back skin of 1-month-old mice. Immunostaining with the NB100-56704 anti-IKKα antibody is showed; similar results were obtained with the H00001147-M04 IKKα antibody (not shown). Note the cytoplasmic expression of the transgene in the C-IKKα mice (D). By contrast, it is located in the nuclei of cells in the N-IKKα mice (E). In both types of transgenic mice the exogenous IKKα is expressed in basal keratinocytes (bk), in the outer root sheath of hair follicles (ORS) & in cells surrounding the sebaceous glands (sb). F. Back skin section of Control mice. The NB100-56704 antibody used does not recognize the endogenous IKKα in immunohistochemical assays. G. Endogenous IKKα expression in control mice using the IKKα (sc-7182) antibody. Scale bar: (C) 70 µm; (D-G) 60 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27121058), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Chen G, Yao Y, Liu Y et Al. IKK?-STAT3-S727 axis: a novel mechanism in DOX-induced cardiomyopathy Cell Mol Life Sci 2024-09-17 [PMID: 39287798]

Qin D, Wang X, Li Y et al. MiR-223-5p and -3p Cooperatively Suppress Necroptosis in Ischemic/Reperfused Hearts. J. Biol. Chem. 2016-08-08 [PMID: 27502281]

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]

Song NY, Li X, Ma B et al. IKK alpha-deficient lung adenocarcinomas generate an immunosuppressive microenvironment by overproducing Treg-inducing cytokines Proceedings of the National Academy of Sciences of the United States of America 2022-02-08 [PMID: 35121655] (IF/IHC, Chemotaxis, WB, Mouse)

Garcia-Garcia V, Alameda J, Page A et al. IKKalpha Induces Epithelial-Mesenchymal Changes in Mouse Skin Carcinoma Cells That Can Be Partially Reversed by Apigenin International Journal of Molecular Sciences 2022-01-25 [PMID: 35163299] (ICC/IF)

Wu Y, Ma L, Cai S et al. RNA-induced liquid phase separation of SARS-CoV-2 nucleocapsid protein facilitates NF-kappa B hyper-activation and inflammation Signal Transduction and Targeted Therapy 2021-12-01 [PMID: 33895773]

Ni J, Guan C, Liu H et al. Ubc13 Promotes K63-Linked Polyubiquitination of NLRP3 to Activate Inflammasome Journal of immunology (Baltimore, Md. : 1950) 2021-04-23 [PMID: 33893171]

Ikkalpha Promotes Intestinal Tumorigenesis by Limiting Recruitment of M1-like Polarized Myeloid Cells. Goktuna SI, Canli O, Bollrath J et al. Cell Rep [PMID: 24882009] (WB, Mouse)

Shi D, Chen M, Liu L et al. Anti-influenza A virus mechanism of three representative compounds from Flos Trollii via TLRs signaling pathways J Ethnopharmacol 2020-01-28 [PMID: 32004628] (WB, Mouse)

Page A, Ortega A, Alameda JP et al. IKK alpha Promotes the Progression and Metastasis of Non-Small Cell Lung Cancer Independently of its Subcellular Localization Comput Struct Biotechnol J. 2019-02-06 [PMID: 30867890] (WB, IHC-P, Mouse)

Liang S, Ma HY, Zhong Z et al. NADPH Oxidase 1 in Liver Macrophages Promotes Inflammation and Tumor Development in Mice Gastroenterology 2019-03-01 [PMID: 30445007] (WB, Mouse)

Balkhi MY, Willette-Brown J, Wittmann G, Hu Y. IKKa deficiency disrupts the development of marginal zone and follicular B cells Genes Immun. 2018-05-08 [PMID: 29740197] (Mouse)

More publications at http://www.novusbio.com/NB100-56704

## Procedures

**Western Blot Protocol for IKK alpha Antibody (NB100-56704)**
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 instructions.

**Immunohistochemistry-Paraffin Protocol for IKK alpha Antibody (NB100-56704)**
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 alpha Antibody (NB100-56704)**
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 alpha Antibody (NB100-56704)**

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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