

# Product Datasheet

## NGFI-B alpha/Nur77/NR4A1 Antibody - BSA Free NB100-56745

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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**NB100-56745**

NGFI-B alpha/Nur77/NR4A1 Antibody - BSA Free

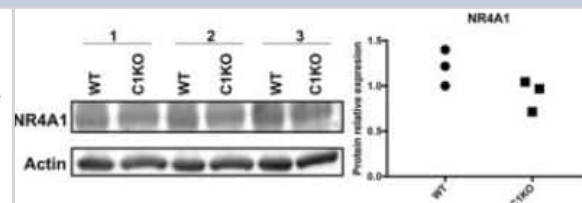
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit NGFI-B alpha/Nur77/NR4A1 Antibody - BSA Free (NB100-56745) is a polyclonal antibody validated for use in IHC, WB, ICC/IF, IP and ChIP. Anti-NGFI-B alpha/Nur77/NR4A1 Antibody: Cited in 31 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	3164
Gene Symbol	NR4A1
Species	Human, Mouse
Immunogen	Partial synthetic peptide made to an internal portion of the human NGFI-B alpha protein (between amino acids 200-300) [UniProt P22736].

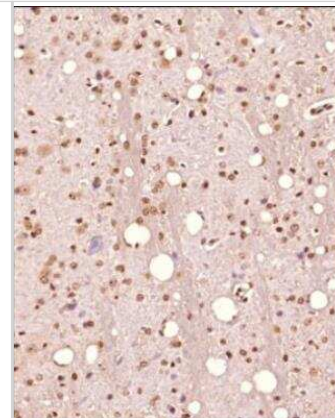
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:500-1:1000, Chromatin Immunoprecipitation 1:20-1:1000. Use reported in scientific literature (Lee et al (2009)), Immunohistochemistry 1:200. Use reported in scientific literature (PMID 31115481), Immunocytochemistry/ Immunofluorescence 1:10-1:2000. Use reported in scientific literature (Chintharlapalli et al (2005)), Immunoprecipitation 1:20-1:1000. Use reported in scientific literature (Ferlini et al (2009)), Immunohistochemistry-Paraffin 1:200, Chromatin Immunoprecipitation (ChIP) 1:20-1:1000

**Images**

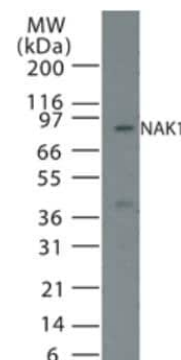
Western Blot: NGFI-B alpha/Nur77/NR4A1 Antibody [NB100-56745] - RNA-seq results were validated by Western blot analysis in WT and calpain-1 KO mice. Western blot images and quantifications showing the expression levels of NGFI-B alpha/Nur77/NR4A1 protein in three independent replicates, respectively. Dot plots were used for the quantification of the expression levels of protein compared to actin control in WT and calpain-1 KO mice. Unpaired t-test in Prism 7 was used to calculate p values, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001. Image collected and cropped by CiteAb from the following publication (<https://www.frontiersin.org/article/10.3389/fgene.2020.00334/full>), licensed under a CC-BY license.



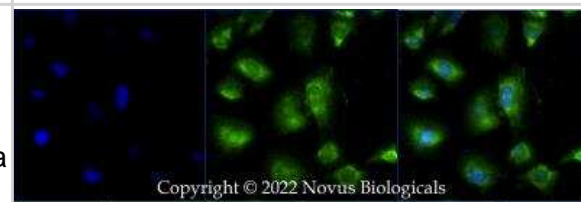
Immunohistochemistry-Paraffin: NGFI-B alpha/Nur77/NR4A1 Antibody [NB100-56745] - Analysis of a FFPE tissue section of mouse brain using 1:200 dilution of NGFI-B alpha/Nur77/NR4A1 antibody (NB100-56745). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



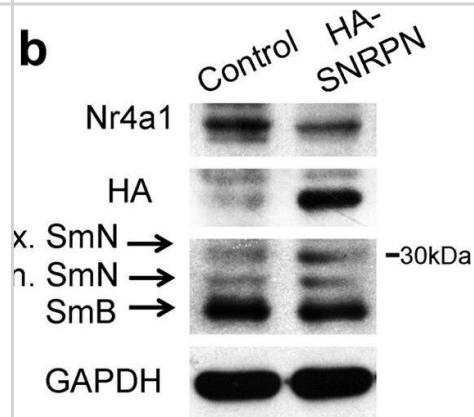
Western Blot: NGFI-B alpha/Nur77/NR4A1 Antibody [NB100-56745] - Analysis of Nur77 (Nak-1) in 15 ugs of NIH3T3 cell lysate using Nur77 antibody at 1:500 dilution



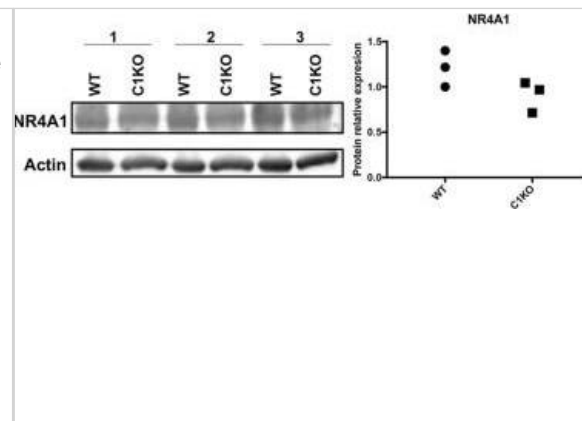
NGFI-B alpha/Nur77/NR4A1 Antibody [NB100-56745] - U-251 MG cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with NGFI-B alpha/Nur77/NR4A1 Antibody (NB100-56745) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



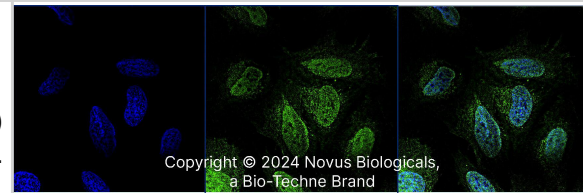
Western Blot: NGFI-B alpha/Nur77/NR4A1 Antibody [NB100-56745] - SNRPN downregulated the expression of Nr4a1. Western blot showed the change in the Nr4a1 protein level by SNRPN overexpression in cultured cortical neurons. Anti-HA antibody was used to detect the effect of HA-tagged SNRPN plasmid. Anti-SNRPN antibody was used to examine exogenous and endogenous SNRPN proteins, including SmN and SmB. The band about 30 kDa represents exogenous HA-tagged SmN. Image collected and cropped by CiteAb from the following publication ([//pubmed.ncbi.nlm.nih.gov/27430727/](https://pubmed.ncbi.nlm.nih.gov/27430727/)) licensed under a CC-BY license.



RNA-seq results were validated by Western blot analysis in WT and calpain-1 KO mice. Western blot images and quantifications showing the expression levels of HSPA1B, DNAJB1, IDE, ARC, PER2, PLA2G4E, and NR4A1 proteins in three independent replicates, respectively. Dot plots were used for the quantification of the expression levels of 7 proteins compared to actin control in WT and calpain-1 KO mice. Unpaired t-test in Prism 7 was used to calculate p values, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



NGFI-B alpha/Nur77/NR4A1 was detected in immersion fixed U-2 OS human osteosarcoma cell line using Rabbit anti-NGFI-B alpha/Nur77/NR4A1 Antigen Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NB100-56745AF488) (green) at 10  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



## Publications

Tsui W, Park Y, Upadhyay S et al. Dual Targeting of Orphan Nuclear Receptors NR4A1 and NR4A2 for Non-Hormonal Endometriosis Therapy. *Endocrinology* 2025-09-26 [PMID: 41002243]

Massman LJ, Pereckas M, Zwagerman NT, Olivier-Van Stichelen S. O-GlcNAcylation Is Essential for Rapid Pomc Expression and Cell Proliferation in Corticotropin Tumor Cells *Endocrinology* 2021-12-01 [PMID: 34418053] (Western Blot, Immunocytochemistry/ Immunofluorescence)

Mandula JK, Sierra-Mondragon RA, Jimenez RV, Chang D et al. Jagged2 targeting in lung cancer activates anti-tumor immunity via Notch-induced functional reprogramming of tumor-associated macrophages *Immunity* 2024-04-18 [PMID: 38636522]

Chen J, Zhang Z, Liu Y et al. Progressive reduction of nuclear receptor Nr4a1 mediates age-dependent cognitive decline *Alzheimers Dement* 2024-04-11 [PMID: 38605605]

Cui C, Wang X, Zheng Y et al. Nur77 as a novel regulator of Paneth cell differentiation and function *Mucosal immunology* 2023-09-06 [PMID: 37683828] (IHC-P, Mouse)

Liu P, Chen Y, Zhang Z et al. Noncanonical contribution of microglial transcription factor NR4A1 to post-stroke recovery through TNF mRNA destabilization *PLoS biology* 2023-07-01 [PMID: 37486903] (IHC, Mouse)

Servaas NH, Hiddingh S, Chouri E et al. Nuclear Receptor Subfamily 4A Signaling as a Key Disease Pathway of CD1c+ Dendritic Cell Dysregulation in Systemic Sclerosis *Arthritis & rheumatology (Hoboken, N.J.)* 2023-02-01 [PMID: 36482877] (ChIP, Human)

Jang S, Zhu J, Qin Q et al. Nur77 Mitigates Endothelial Dysfunction Through Activation of Both Nitric Oxide Production and Anti-Oxidant Pathways *SSRN* 2023-06-27 (WB, Human)

Yuan R, Zhang W, Nie P et al. Nur77 Deficiency Exacerbates Macrophage NLRP3 Inflammasome-Mediated Inflammation and Accelerates Atherosclerosis *Oxidative medicine and cellular longevity* [PMID: 35464766] (Chemotaxis, Mouse)

Jiang C, He J, Xu S et al. NR4A1 promotes LEF1 expression in the pathogenesis of papillary thyroid cancer *Cell death discovery* 2022-02-02 [PMID: 35110542] (Chemotaxis, IF/IHC, Human)

Guo H, Golczer G, Wittner BS et al. NR4A1 regulates expression of immediate early genes, suppressing replication stress in cancer *Molecular cell* 2021-10-07 [PMID: 34624217] (IP, Human)

Bruno NE, Nwachukwu JC, Hughes DC et al. Activation of Crtc2/Creb1 in skeletal muscle enhances weight loss during intermittent fasting *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2021-12-01 [PMID: 34748223] (WB, Mouse)

More publications at <http://www.novusbio.com/NB100-56745>



## Procedures

### Western Blot Protocol for NGFI-B alpha/Nur77/NR4A1 Antibody (NB100-56745)

#### 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.

### Immunocytochemistry/Immunofluorescence Protocol for NGFI-B alpha/Nur77/NR4A1 Antibody (NB100-56745)

#### 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. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization 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.

### Immunohistochemistry-Paraffin Protocol for NGFI-B alpha/Nur77/NR4A1 Antibody (NB100-56745)

#### 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.



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### Products Related to NB100-56745

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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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