

# Product Datasheet

## GAP-43 Antibody - BSA Free

### NB300-143

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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**NB300-143**

GAP-43 Antibody - BSA Free

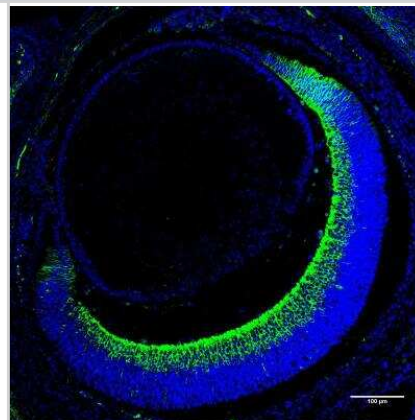
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.035% Sodium Azide
Purity	Immunogen affinity purified
Buffer	50% PBS, 50% glycerol
Target Molecular Weight	43 kDa

Product Description	
Host	Rabbit
Gene ID	2596
Gene Symbol	GAP43
Species	Human, Mouse, Rat, Porcine, Bovine, Canine, Chicken, Drosophila, Equine, Primate
Reactivity Notes	Reactivity to Canine, Chicken, and Primate reported in scientific literature (PMID: 30647968, 30819546, and 24249398 respectively). Drosophila reactivity reported in scientific literature (PMID: 30819546).
Marker	Neuronal Marker
Immunogen	C-terminal peptide of rodent GAP43, KEDPEADQEHA coupled to KLH.

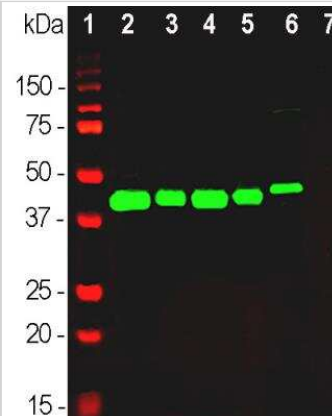
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:10000, Flow Cytometry, Immunohistochemistry 1-2 ug/ml, Immunocytochemistry/ Immunofluorescence 1:1000, Immunohistochemistry-Paraffin 1-2 ug/ml, Immunohistochemistry-Frozen 1-2 ug/ml
Application Notes	This GAP43 antibody is useful for Immunocytochemistry/Immunofluorescence, and Western blot, where it recognizes a band at 43 kDa. Use in IHC-P and IHC-Fr reported in scientific literature (PMID: 29950987 and 27007292 respectively). Use in FLOW reported in scientific literature (PMID: 21614130).

**Images**

Immunohistochemistry-Paraffin: GAP-43 Antibody [NB300-143] - Review image from confirmed customer on mouse E15.5 paraffin sections.



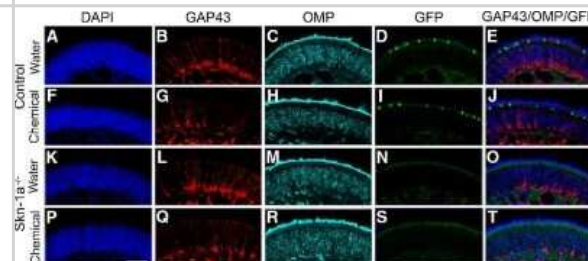
**Western Blot: GAP-43 Antibody [NB300-143]** - Different tissue and cell lysates using rabbit pAb to GAP43, dilution 1:20,000 in green: [1] protein standard (red), [2] rat brain, [3] rat spinal cord, [4] mouse brain, [5] mouse spinal cord, [6] SH-SY5Y cells, [7] C6 cells. Single band at 43 kDa mark corresponds to GAP43 protein. The GAP43 protein is detected only in the lysates of neuronal origin. C6 cells are a rat glioma cell line and do not express GAP43 protein



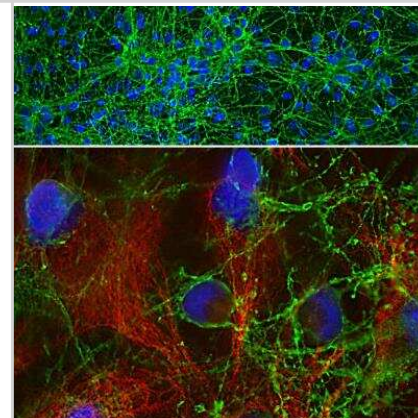
**Immunohistochemistry: GAP-43 Antibody [NB300-143]** - Representative images of immunohistological staining (brown) of OMP-positive (OMP+) cells, GAP43+ immature ORNs. Tissue sections were counterstained with the nuclear dye hematoxylin (blue). Numbers of SOX2+ ORN progenitors and Ki67+ actively proliferating cells per mm of the basal layer and OMP+ mature ORNs, GAP43+ immature ORNs, and Cas3+ apoptotic cells per mm of the OE in saline or rhIGF-1-treated mice. Open circles, rectangles, and triangles represent the values for each mouse in the saline, low-IGF-1, and high-IGF-1 treated groups (each n = 6), respectively. Image collected and cropped by Citeab from the following publication (Dose-Dependent Effects of Insulin-Like Growth Factor 1 in the Aged Olfactory Epithelium. *Front Aging Neurosci* (2018)) licensed under a CC-BY license.



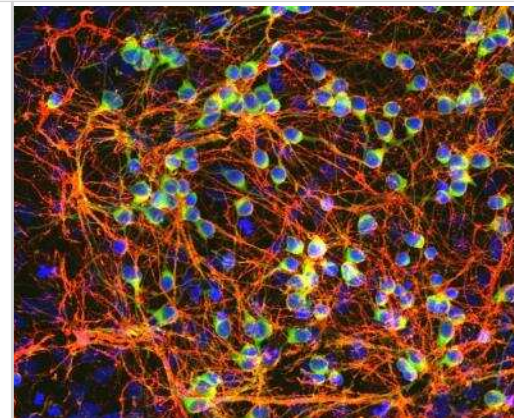
**Immunohistochemistry-Paraffin: GAP-43 Antibody [NB300-143]** - Posterior MOE sections at approximately the same region where EOG was recorded were obtained from control and Skn-1a<sup>-/-</sup> mice exposed to either water or chemicals for 2 wks. The sections were immunoreacted with antibodies against GAP43 and OMP and stained with DAPI. Confocal images were taken from olfactory turbinate II. Similar morphology and marker expression were found in four groups of mice, indicating there was no obvious tissue damage in both control and Skn-1a<sup>-/-</sup> mice after chemical exposure in the posterior MOE regions where EOG recordings were performed. Scale bar: 50  $\mu$ m. Image collected and cropped by CiteAb from the following publication (<https://eneuro.sfn.org/lookup/doi/10.1523/ENEURO.0135-17.2017>), licensed under a CC-BY license.



**Immunocytochemistry/Immunofluorescence: GAP-43 Antibody [NB300-143]** - Cortical neuron-glia cell culture from E20 rat stained with rabbit pAb to GAP43, dilution 1:2,000 in green, and costained with mouse mAb to vimentin, dilution 1:2,000, in red. Blue: DAPI staining of nuclear DNA. GAP43 antibody labels protein expressed in the axonal membrane of neuronal cells, while vimentin antibody stains intermediate filaments in fibroblasts and other non-neuronal cells.



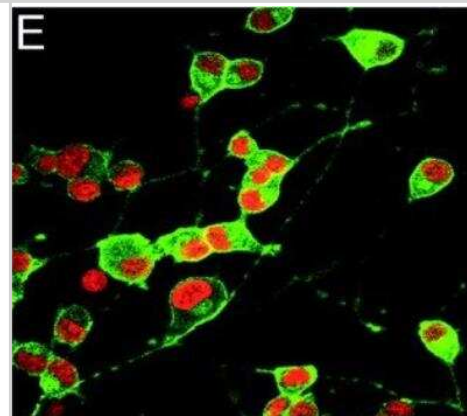
Immunocytochemistry/Immunofluorescence: GAP-43 Antibody [NB300-143] - Rat E18 mixed neuron/glia cultures with rabbit GAP43 (red) and 5B10, mouse monoclonal to MAP-tau (green).



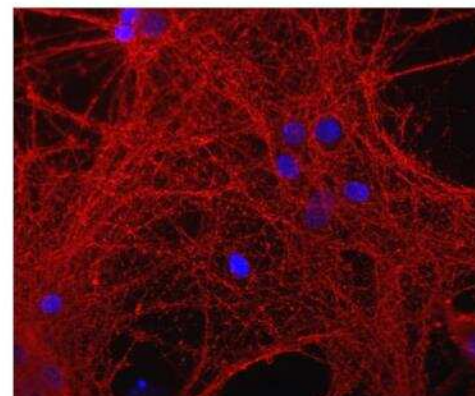
Western Blot: GAP-43 Antibody [NB300-143] - Western blots of homogenate of cow cerebellum stained with RPCA-GAP-43 . A prominent band running at ~43kDa represents the full length GAP-43.



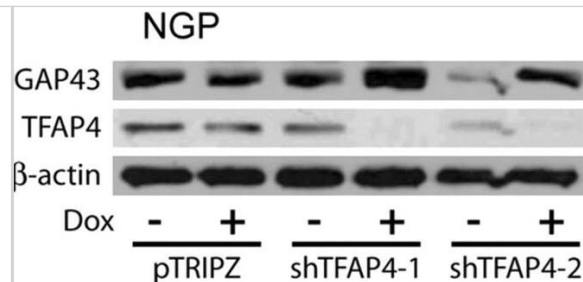
Immunocytochemistry/Immunofluorescence: GAP-43 Antibody [NB300-143] - Immunofluorescence of GAP-43 (green), a molecular marker of neurite outgrowth, demonstrates intense staining in overexpressing wild-type PS-1 (E) PC-12 cells. (Teo, et al, 2005)



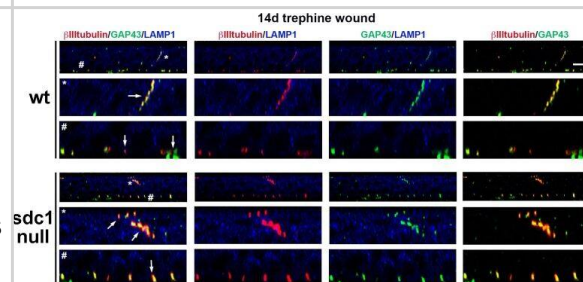
Immunocytochemistry/Immunofluorescence: GAP-43 Antibody [NB300-143] - Mixed neuron-glia cultures stained with RPCA-GAP43 (red), blue is DNA staining.



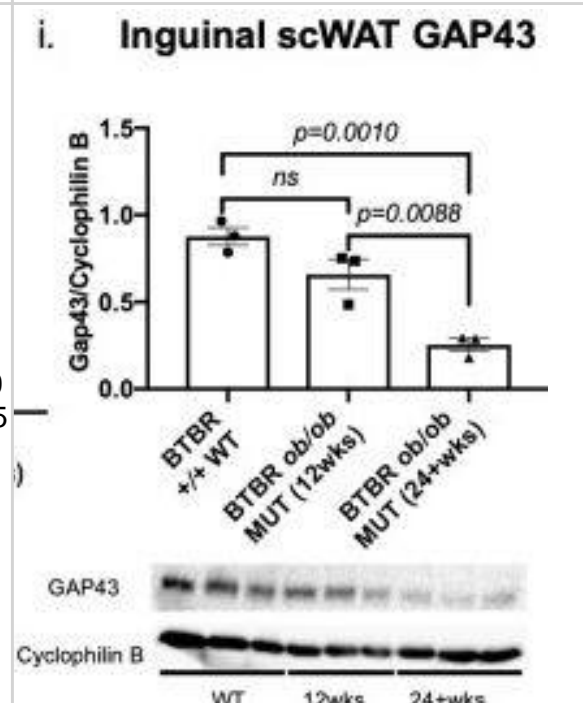
Western Blot: GAP-43 Antibody [NB300-143] - Silencing TFAP4 induces neuroblastoma cell differentiation. a Morphology of neuroblastoma cells after silencing TFAP4. Cells were grown in culture for 8 days, with 1  $\mu\text{g/ml}$  doxycycline (shRNA induced) or without doxycycline. b Neurites # per field, & neurite length (pixels) were quantified with or without doxycycline. Mean  $\pm$  std dev. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , \*\*\*\*,  $P < 0.0001$ . c GAP43 expression level (qRT-PCR) & protein level (western blot) in neuroblastoma cells. Gene expression was measured by quantitative PCR 4 days after induction of shRNA against TFAP4. Protein samples for western blot were collected from MYCN-amplified cells that were cultured with or without doxycycline (1  $\mu\text{g/ml}$ ) for 6 days Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/29880876>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



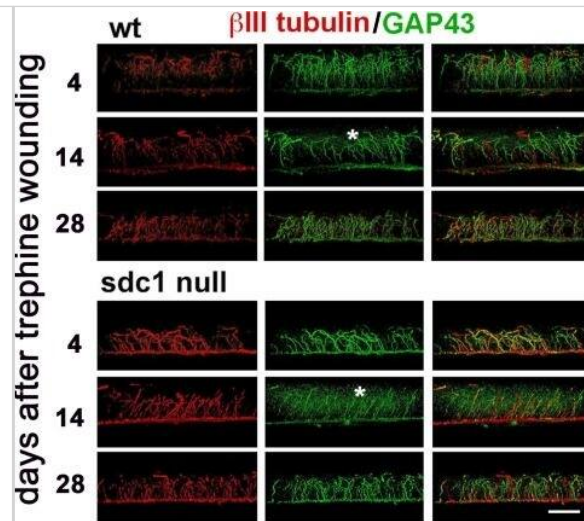
Immunocytochemistry/ Immunofluorescence: GAP-43 Antibody [NB300-143] - LAMP1 increases in SDC-null corneas 14 days after trephine wound. Representative 3D images rotated to generate cross-sectional views show the localization of GAP43 (green),  $\beta$ III tubulin (red), & LAMP1 (blue) in WT (top) & SDC1-null (bottom) corneas. These images show staining within a 0.5- $\mu\text{m}$  cross-sections. Data indicate that axon fragments are being degraded (arrows). Representative INTs (\*) & SBNs (#) have been highlighted & enlarged 3-fold. When LAMP1 expression within apical & basal cell layers 14 days after trephine injury is quantified & compared with unwounded controls (Fig. 2B), LAMP1 increases over 3-fold in SDC1-null corneas but remains the same in WT corneas. Scale bar: 10  $\mu\text{m}$ . Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28973369>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



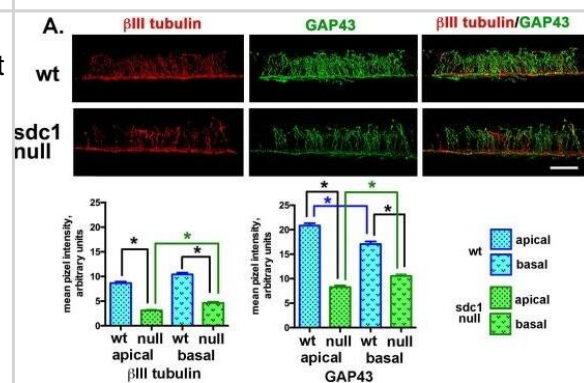
Western Blot: GAP-43 Antibody [NB300-143] - Obesity & diabetes led to white adipose tissue neuropathy. The BTBR ob/ob (MUT) model of obesity & diabetes was compared to BTBR +/+ wild-type (WT) for body weight measurements (a), & adiposity (b). Body & tissue weight data were analyzed by two-tailed Student's t-test. Von Frey tactile allodynia analysis was performed on MUT & WT animals to determine onset of peripheral neuropathy (c). Von Frey data was analyzed by ANOVA with Sidak's post hoc test. For (a-c), all males; WT N = 8, 12–20 weeks old; MUT N = 6, 12–24 weeks old. Protein levels of PGP9.5 (d), as well as TH (e) in inguinal scWAT of the MUT & WT mice were measured by western blotting. For (e), lane 5 was excluded from analyses due to uneven resolution of housekeeper. For (d-e), all males; WT N = 5, 12–20 weeks old & MUT N = 4, 12–20 weeks old. Protein expression of PGP9.5 (f), TH (g), PSD95 (h), & GAP43 (i) in inguinal scWAT of 12–25 weeks old WT, 12 week old MUT & 24–28 week old MUT was measured by western blotting. All males, N = 3 per group. Western blot data were normalized to either  $\beta$ -Tubulin or Cyclophilin B, band intensity were quantified in Image J, & analyzed by two-tailed Student's t-test. Error bars are SEMs. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31509546>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: GAP-43 Antibody [NB300-143] - Intraepithelial nerve terminals extension into the apical most cell layers increases in SDC1-null corneas after trephine injury. Representative 3D images rotated to generate cross-sectional views show the localization of  $\beta$ III tubulin (red) & GAP43 (green) at 4, 14, & 28 days after trephine wounding in WT & SDC1-null corneas. Punctate GAP43 is increased within the epithelium in SDC1-null compared with WT corneas at 14 days (\*).  $\beta$ III tubulin+ & GAP43+ INTs in the apical most cell layers were quantified as a function of time after trephine injury & normalized relative to control (presented in Fig. 2A). Asterisks within bars indicate differences that are significant relative to unwounded controls; asterisks between bars indicate significant differences between time points. In WT corneas, the numbers of  $\beta$ III tubulin+ & GAP43+ INTs extending apically decrease relative to controls at 28 days for  $\beta$ III tubulin + INTs & at 4 & 28 days for GAP43+ INTs, in SDC1-null corneas, both  $\beta$ III tubulin+ & GAP43+ INTs increase at all time points assessed. Scale bar: 25  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28973369>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: GAP-43 Antibody [NB300-143] - Differences in INTs & LAMP1 localization in WT & SDC1-null adult corneas. Representative high-resolution confocal 3D images rotated as indicated in the cartoon to the left were used to generate cross sectional views to show the localization of  $\beta$ III tubulin (red) & GAP43 (green) in intraepithelial nerve terminals in control WT & SDC1-null corneas. The images shown in (A) project through 135  $\mu$ m of tissue, images in (B) project through 0.5  $\mu$ m of tissue. (A) Intraepithelial corneal nerves are shown to be linear, arise from the subbasal nerves (SBNs), & project apically. Quantitation of  $\beta$ III tubulin & GAP43 within apical & basal layers of the corneal epithelium reveals that WT corneas have significantly more INTs in both basal & apical cell layers compared with SDC1-null corneas. Although SDC1-null corneas have fewer INTs, they are equally capable of extending toward the apical most cell layers. (B) In the 0.5- $\mu$ m cross-sectional slices through the tissue, INTs appear discontinuous. In addition to  $\beta$ III tubulin & GAP43, LAMP1 is shown in blue. LAMP1 is a lysosomal marker; co-localization of GAP43 and/or  $\beta$ III tubulin within corneal epithelial cell lysosomes indicate that axon fragments have been phagocytosed & are being degraded (arrows). The sites indicated by the asterisks have been digitally enlarged 3-fold. LAMP1 was quantified in WT & SDC1-null corneas. Data show that cells within the apical layers express more LAMP1 compared with basal cells in both genotypes of mice. In addition, WT corneas express 2- to 3-fold more LAMP1 in apical & basal layers compared with SDC1-null corneas. Scale bar: 10  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28973369>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Lemons K, Fu Z, Ogura T, Lin W TRPM5-expressing Microvillous Cells Regulate Region-specific Cell Proliferation and Apoptosis During Chemical Exposure *Neuroscience* 2020-03-26 [PMID: 32224228]

Mulc D, Smilovi? D, Krsnik , Junakovi?-Munjas A et Al. Fetal development of the human amygdala *J Comp Neurol* 2024-01-30 [PMID: 38289194]

Olguin SL, Patel P, Buchanan CN et al. KHSRP loss increases neuronal growth and synaptic transmission and alters memory consolidation through RNA stabilization *Communications biology* 2022-07-07 [PMID: 35798971]

Singh HP, Shayler DWH, Fernandez GE et Al. An immature, dedifferentiated, and lineage-deconstrained cone precursor origin of N-Myc-initiated retinoblastoma *Proc Natl Acad Sci U S A* 2022-07-08 [PMID: 35867756]

Merle-Nguyen L, Ando-Grard O, Bourgon C et Al. Early corticosteroid treatment enhances recovery from SARS-CoV-2 induced loss of smell in hamster *Brain Behav Immun* 2024-04-10 [PMID: 38367845]

Cavarischia-Rega C, Sharma K, Fitzgerald JC, Macek B. Proteome Dynamics in iPSC-Derived Human Dopaminergic Neurons *Molecular & Cellular Proteomics : MCP* 2024-09-07 [PMID: 39251023]

Hara Y, Jha MK, Huang JY et al. The IL-4-IL-4R $\alpha$  axis modulates olfactory neuroimmune signaling to induce loss of smell. *Allergy* 2024-10-17 [PMID: 39418114]

Ueha R, Ito T, Furukawa R et al. Oral SARS-CoV-2 Inoculation Causes Nasal Viral Infection Leading to Olfactory Bulb Infection: An Experimental Study *Frontiers in Cellular and Infection Microbiology* 2022-06-13 [PMID: 35770069]

Mori E, Ueha R, Kondo K et al. Squamous and Respiratory Metaplasia After Olfactory Mucosal Resection *Frontiers in Neuroscience* 2021-07-20 [PMID: 34354563] (Immunohistochemistry)

Ueha R, Ito T, Ueha S et al. Evidence for the spread of SARS-CoV-2 and olfactory cell lineage impairment in close-contact infection Syrian hamster models *Frontiers in Cellular and Infection Microbiology* 2022-10-21 [PMID: 36339331]

Matthew D. Cain, N. Rubin Klein, Xiaoping Jiang, Hamid Salimi, Qingping Wu, Mark J. Miller, William B. Klimstra, Robyn S. Klein Post-exposure intranasal IFN $\alpha$  suppresses replication and neuroinvasion of Venezuelan Equine Encephalitis virus within olfactory sensory neurons *Journal of Neuroinflammation* 2024-01-17 [PMID: 38233868]

Magdalena Blaszkiewicz, Tianyi Tao, Kofi Mensah-Arhin, Jake W Willows, Rhiannon Bates, Wei Huang, Lei Cao, Rosemary L Smith, Kristy L Townsend Gene therapy approaches for obesity-induced adipose neuropathy: Device-targeted AAV-mediated neurotrophic factor delivery to adipocytes in subcutaneous adipose. *Molecular therapy : the journal of the American Society of Gene Therapy* 2024-03-01 [PMID: 38429927]

More publications at <http://www.novusbio.com/NB300-143>

## Procedures

### Protocol specific for GAP43 Antibody (NB300-143)

#### IHC-FFPE sections

##### I. Deparaffinization:

- A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
- B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

##### II. Quench Endogenous Peroxidase:

- A. Place slides in peroxidase quenching solution: 15-30 minutes. To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.  
Use within 4 hours of preparation
- B. Place slides in distilled water: 2 changes for 2 minutes each.

##### III. Retrieve Epitopes:

- A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celsius.
- B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
- C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
- D. Slowly add distilled water to further cool for 5 minutes.
- E. Rinse slides with distilled water. 2 changes for 2 minutes each.

##### IV. Immunostaining Procedure:

- A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
- B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
- C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
- D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
- E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
- G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
- I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
- K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
- L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
- M. Rinse slides in distilled water.
- N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
- O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
- P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
- Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
- R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
- S. Lay slides on a flat surface to dry prior to viewing under microscope.

##### NOTES:

- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60 degrees Celsius oven.



-All steps in which Xylene is used should be performed in a fume hood.

-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.

-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.

-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.

-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).



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General: novus@novusbio.com

### **Products Related to NB300-143**

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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NB300-143B	GAP-43 Antibody [Biotin]
NBP2-53033-20ug	Recombinant Human GAP-43 His Protein

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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.

For more information on our 100% guarantee, please visit [www.novusbio.com/guarantee](http://www.novusbio.com/guarantee)

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