

# Product Datasheet

## TGN46 Antibody - BSA Free

### NBP1-49643

Unit Size: 0.1 ml

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

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**NBP1-49643**

TGN46 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
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

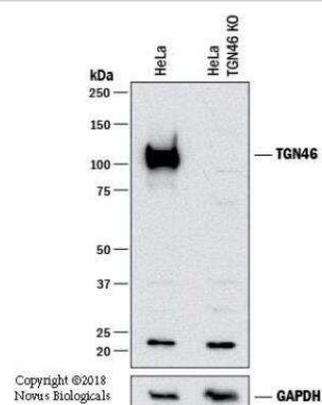
Product Description	
Description	Novus Biologicals Knockout (KO) Validated Rabbit TGN46 Antibody - BSA Free (NBP1-49643) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-TGN46 Antibody: Cited in 36 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	10618
Gene Symbol	TGOLN2
Species	Human, Bovine, Monkey
Reactivity Notes	Monkey reactivity reported in scientific literature (PMID: 30135710). Bovine reactivity reported in scientific literature (PMID: 30135710).
Marker	TGN Marker
Immunogen	Partial recombinant protein made to an internal region of the human TGN46 protein (within residues 200-350). [Swiss-Prot: O43493]
Notes	Manufactured by Genomic Antibody Technology™. GAT <a href="#">FAQs</a>

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Knockout Validated
Recommended Dilutions	Western Blot 2ug/mL, Flow Cytometry, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 31455601), Immunohistochemistry-Paraffin 1:400, Immunoblotting reported in scientific literature (PMID 25410859), Flow (Intracellular), Knockout Validated
Application Notes	Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

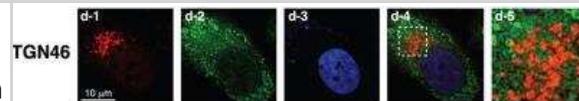


## Images

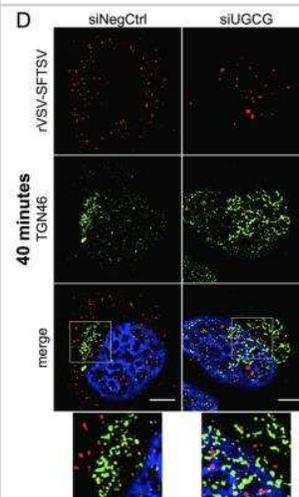
**Western Blot: TGN46 Antibody [NBP1-49643]** - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and TGN46 knockout (KO) HeLa cell line. PVDF membrane was probed with 1:1000 of Rabbit Anti-Human TGN46 Polyclonal Antibody (NBP1-49643) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (HAF008). Specific band was detected for TGN46 at approximately 100 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.



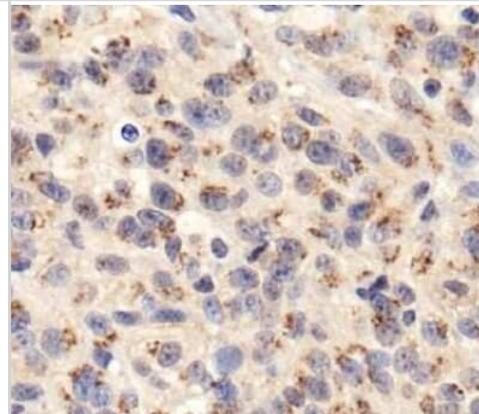
**Immunocytochemistry/Immunofluorescence: TGN46 Antibody [NBP1-49643]** - A7 cells expressing F-NTPase were fixed and dual stained with an anti-FLAG antibody (green) and TGN46 antibody (red). Colocalization of F-NTPase with the signals of organelle-specific markers are shown in yellow (arrows). The dashed boxes in each merged image were enlarged and are shown at the right. As noted, the nonvesicular areas of F-NTPase does not colocalize with the signals of TGN46 (a trans-Golgi marker). Scale bars, 10  $\mu$ m. Image collected and cropped by CiteAb from the following publication (<https://jvi.asm.org/content/92/5/e01824-17>) licensed under a CC-BY license.



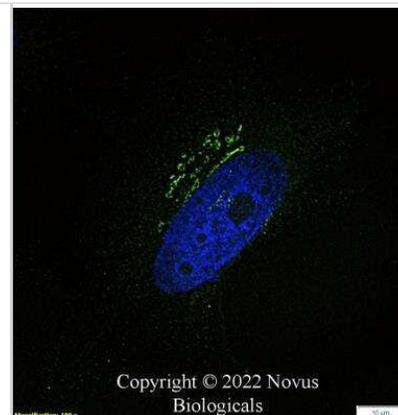
**Immunocytochemistry/Immunofluorescence: TGN46 Antibody [NBP1-49643]** - U-2 OS cells were transfected with siRNAs targeting UGCG (siUGCG) or a non-targeting control (siNegCtrl) and plated onto glass coverslips. At 72 hours post-transfection cells were chilled to 4 degrees C on ice and rVSV-SFTSV was bound by centrifugation (1200xg, 30', 4 degrees C). Following centrifugation, media was replaced with pre-warmed media (37 degrees C) and the cells placed in a 37 degrees C incubator for 40 minutes before fixation in 1% paraformaldehyde for 15 minutes. Cells were then immunostained for viral antigen (anti-VSV M, red), TGN46 (green), and nuclei stained with DAPI (blue). Images are representative from at least 3 independent experiments. Boxes indicate zoomed-in regions. Scale bar represents 5 $\mu$ m. Image collected and cropped by CiteAb from the following publication (<https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006316>) licensed under a CC-BY license.



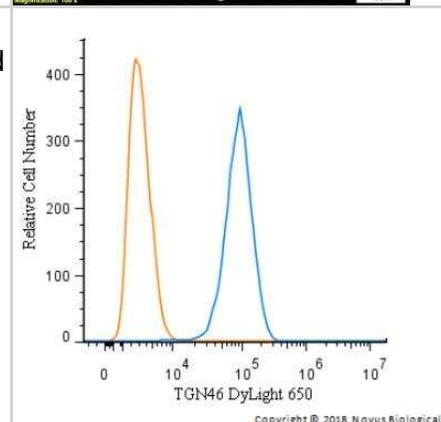
**Immunohistochemistry: TGN46 Antibody [NBP1-49643]** - Analysis of TGOLN2 in human renal cell carcinoma.



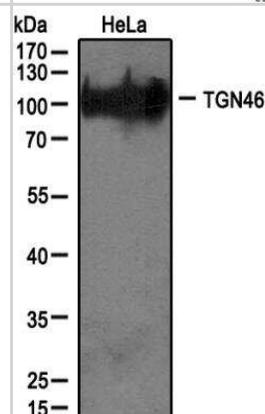
**Immunocytochemistry/Immunofluorescence: TGN46 Antibody [NBP1-49643]** - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with TGN46 Antibody conjugated to Alexa Fluor 488 (NBP1-49643AF488) at 2 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



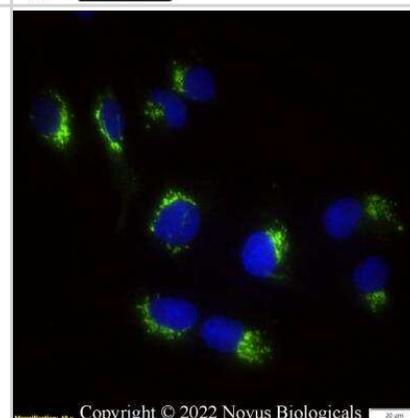
**Flow Cytometry: TGN46 Antibody [NBP1-49643]** - An intracellular stain was performed on HepG2 cells with NBP1-49643C (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 650.



**Western Blot: TGN46 Antibody [NBP1-49643]** - Analysis of extracts from HeLa cells using TGN46 antibody (NBP1-49643, 1:100).



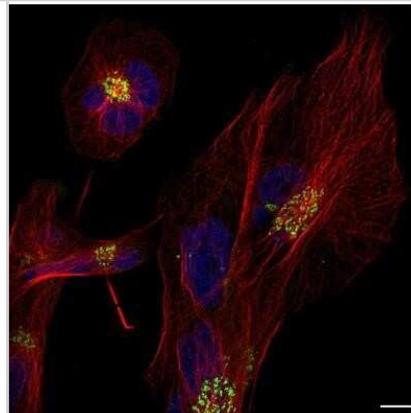
**Immunocytochemistry/Immunofluorescence: TGN46 Antibody [NBP1-49643]** - HepG2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with TGN46 Antibody conjugated to Alexa Fluor 488 (NBP1-49643AF488) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



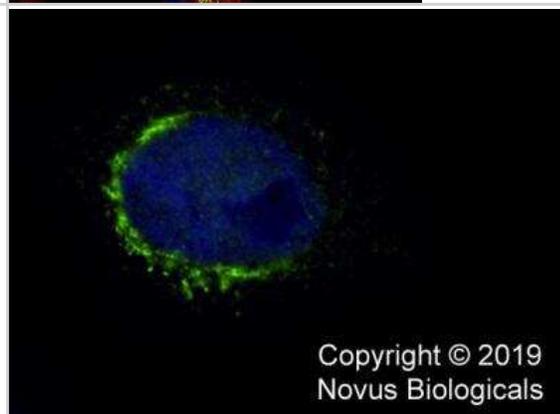
Immunocytochemistry/Immunofluorescence: TGN46 Antibody [NBP1-49643] - Analysis of A549 cells using TGN46 antibody (NBP1-49643, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).



Immunocytochemistry/Immunofluorescence: TGN46 Antibody [NBP1-49643] - T98G glioblastoma cells probed for Golgi (Alexa Fluor 488 conjugated TGN-46 antibody, Green), tubulin (Alexa Fluor 594, Red), and nucleus (DAPI, blue). Image from the Alexa Fluor 488 version of this antibody. Image from verified customer review.



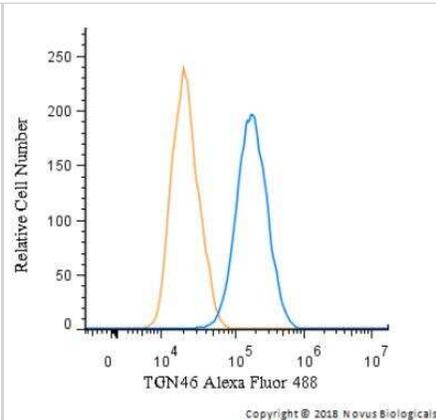
Immunocytochemistry/Immunofluorescence: TGN46 Antibody [NBP1-49643] - HeLa cells were fixed in 4% paraformaldehyde for 10 min and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- at 2 ug/ml for 60 minutes at room temperature and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective.



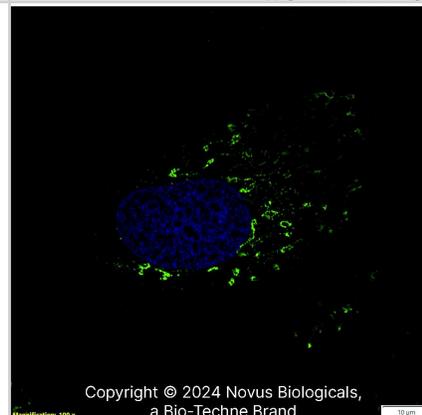
Flow (Intracellular): TGN46 Antibody [NBP1-49643] - An intracellular stain was performed on U-937 cells with NBP1-49643 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by rabbit IgG APC-conjugated secondary antibody (F0111, R&D Systems).



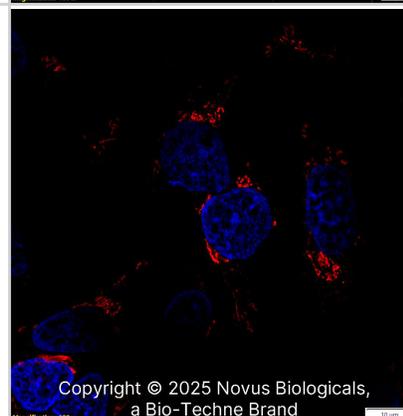
Flow (Intracellular): TGN46 Antibody [NBP1-49643] - An intracellular stain was performed on HepG2 cells with TGN46 Antibody NBP1-49643AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10  $\mu\text{g}/\text{mL}$  for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



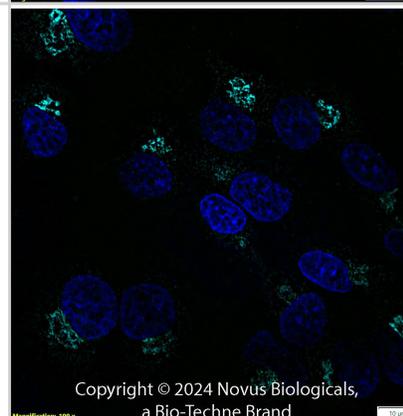
TGN46 was detected in immersion fixed Caco-2 human colorectal adenocarcinoma cell line using Rabbit anti-TGN46 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP1-49643AF488) (green) at 2  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



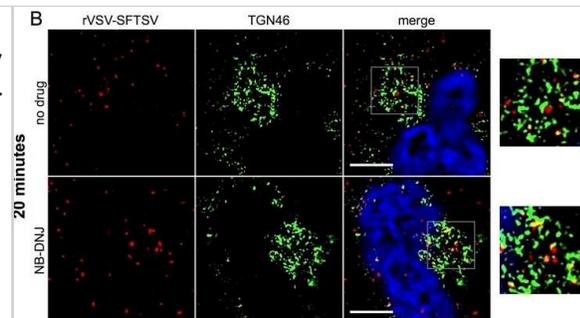
TGN46 was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rabbit anti-TGN46 Affinity Purified Polyclonal Antibody conjugated to Biotin (Catalog # NBP1-49643B) at 2  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



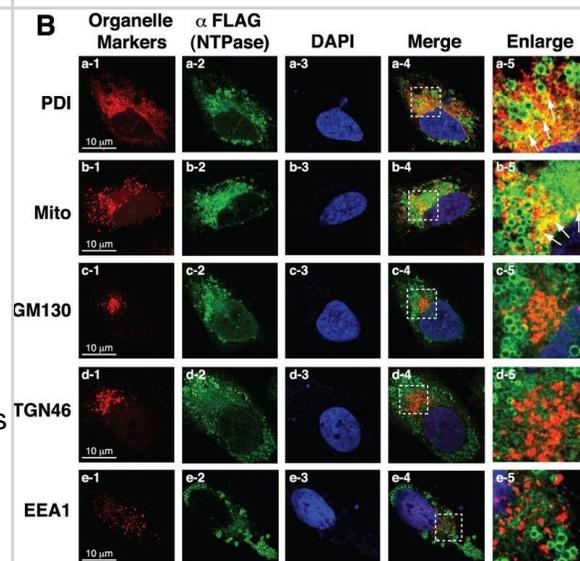
TGN46 was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rabbit anti-TGN46 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP1-49643AF647) (light blue) at 2  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.



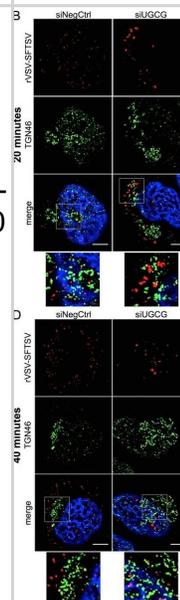
Immunofluorescence microscopy of incoming virus particles, 20 minutes. (A, B) A549 cells were plated onto glass coverslips and the following day replaced with media containing NB-DNJ (200 $\mu$ M) or left untreated. Forty-eight hours later, cells were chilled to 4C on ice, then rVSV-SFTSV was bound by centrifugation (1200xg, 30', 4C). Following centrifugation, media was replaced with pre-warmed media (37C) and the cells placed in a 37C incubator for 20 minutes before fixation in 2% paraformaldehyde for 10 minutes. Cells were then immunostained for viral antigen (anti-VSV M, red), cellular markers (green), and nuclei stained with DAPI (blue). Images are representative from at least 3 independent experiments. (A) A549 cells co-stained for rVSV-SFTSV (red) and early endosome marker EEA1 (green). (B) A549 cells co-stained for rVSV-SFTSV (red) and trans-Golgi marker TGN46 (green). Boxes indicate zoomed-in regions. Scale bar represents 5 $\mu$ m. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/28388693>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



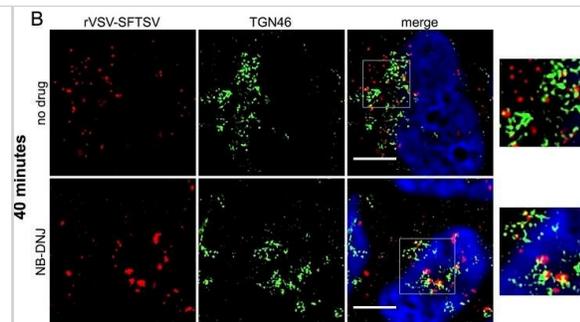
Nonvesicular fraction of GII-NTPase significantly localizes to the ER or mitochondria in cells. (A) The expression plasmid encoding F-NTPase was transfected into A7 cells. After 24 h of transfection, cells were fixed and dual labeled with an anti-FLAG antibody (green) and a rabbit polyclonal anti-NTPase antibody (red). Colocalization of signals recognized by the anti-FLAG and the anti-NTPase antibodies was detected as yellow color. Scale bar, 50  $\mu$ m. (B) A7 cells expressing F-NTPase were fixed and dual stained with an anti-FLAG antibody (green) and a set of antibodies or a fluorescent dye (MitoTracker [Mito]) specific for organelle markers (red). Colocalization of F-NTPase with the signals of organelle-specific markers are shown in yellow (arrows). The dashed boxes in each merged image were enlarged and are shown at the right. As noted, the nonvesicular areas of F-NTPase colocalize with the signals of PDI (an ER marker) or Mito (a mitochondrial probe) but not those of GM130 (a cis-Golgi marker), TGN46 (a trans-Golgi marker), and EEA1 (an endosomal marker). Scale bars, 10  $\mu$ m. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/29212938>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunofluorescence microscopy of rVSV-SFTSV particles following UGCG knockdown. (A-D) U-2 OS cells were transfected with siRNAs targeting UGCG (siUGCG) or a non-targeting control (siNegCtrl) and plated onto glass coverslips. At 72 hours post-transfection cells were chilled to 4C on ice and rVSV-SFTSV was bound by centrifugation (1200xg, 30', 4C). Following centrifugation, media was replaced with pre-warmed media (37C) and the cells placed in a 37C incubator for 20 or 40 minutes before fixation in 1% paraformaldehyde for 15 minutes. Cells were then immunostained for viral antigen (anti-VSV M, red), cellular markers (green), and nuclei stained with DAPI (blue). Images are representative from at least 3 independent experiments. (A,B) U-2 OS cells fixed after 20 minutes were co-stained for rVSV-SFTSV (red) and EEA1 (A) or TGN46 (B) (green). (C,D) U-2 OS cells fixed after 40 minutes and stained as above. Boxes indicate zoomed-in regions. Scale bar represents 5 $\mu$ m. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/28388693>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunofluorescence microscopy and quantification of incoming virus particles. (A, B) A549 cells prepared as described in Fig 8 were fixed 40 minutes post-warming and co-stained for rVSV-SFTSV (red) and the early endosome marker EEA1 (green) (A) or TGN46 (B). Cells were treated with NB-DNJ (bottom panels) or left untreated (top panels). Boxes indicate zoomed-in regions. Scale bar represents 5 $\mu$ m. (C, D) Quantitative image analysis was performed to measure the volume of discrete VSV M-stained puncta within z-stack images in untreated and NB-DNJ treated cells at both 20 and 40 minutes post-warming. Puncta were counted for at least 6 independent z-stacks per sample for both rVSV-SFTSV (C) and VSV (D) infected cells. (\*\*\*\*  $p < 0.0001$  using Welch's one-tailed t-test). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/28388693>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Yen JB, Wei LH, Chen LW et al. Subcellular localization and functional characterization of GII.4 norovirus-encoded NTPase. *J. Virol.* 2017-12-06 [PMID: 29212938] (Immunocytochemistry/ Immunofluorescence, Human)

Arachchige SP, Henke W, Pramanik A et al. Analysis of Select HSV-1 Proteins for Restriction of Human Immunodeficiency Virus Type 1: The HSV-1 gM Protein Potently Restricts HIV-1 by Preventing the Intracellular Transport and Processing of Env gp160 *J. Virol.* 2017-11-01 [PMID: 29093081] (Immunocytochemistry/ Immunofluorescence, Human)

Miller AN, Houlihan PR, Matamala E et al. The SARS-CoV-2 accessory protein Orf3a is not an ion channel, but does interact with trafficking proteins *eLife* 2023-01-25 [PMID: 36695574] (Immunocytochemistry/ Immunofluorescence, Human)

Read CB, Ali AN, Stephenson DJ, Macknight HP et al. Ceramide-1-phosphate is a regulator of Golgi structure and is co-opted by the obligate intracellular bacterial pathogen *Anaplasma phagocytophilum* *mBio* 2024-02-28 [PMID: 38415594]

Han S, Ye X, Yang J, Peng X et al. Host specific sphingomyelin is critical for replication of diverse RNA viruses *Cell Chem Biol* 2024-11-20 [PMID: 39566509]

Iwahashi N, Ikezaki M, Matsuzaki I, Yamamoto M et al. Calreticulin Regulates Syncytialization Through Control of the Synthesis and Transportation of E-Cadherin in BeWo Cells *Endocrinology* 2018-12-15 [PMID: 30551188]

Ko PJ, Woodrow C, Dubreuil MM et al. A ZDHHC5-GOLGA7 Protein Acyltransferase Complex Promotes Nonapoptotic Cell Death *Cell Chem Biol* 2019-10-17 [PMID: 31631010]

Rutger D, Luteijn, Sypke R. van Terwisga, Jill E. Ver Eecke, Liberty Onia, Shivam A. Zaver, Joshua J Woodward, Richard W Wubbolts, David H. Raulet, Frank J.M. van Kuppeveld The activation of the adaptor protein STING depends on its interactions with the phospholipid PI4P *Science signaling* 2024-04-04 [PMID: 38470955]

Hein LK, Apaja PM, Hattersley K et al. A novel fluorescent probe reveals starvation controls the commitment of amyloid precursor protein to the lysosome. *Biochim. Biophys. Acta.* 2017-06-19 [PMID: 28641977]

Hans C. Leier, Jules B. Weinstein, Jennifer E. Kyle, Joon-Yong Lee, Lisa M. Bramer, Kelly G. Stratton, Douglas Kempthorne, Aaron R. Navratil, Endale G. Tafesse, Thorsten Hornemann, William B. Messer, Edward A. Dennis, Thomas O. Metz, Eric Barklis, Fikadu G. Tafesse A global lipid map defines a network essential for Zika virus replication *Nature Communications* 2020-07-21 [PMID: 32694525]

Gu Y, Princely Y, Kumar S et al. Mammalian Atg8 proteins regulate lysosome and autolysosome biogenesis through SNAREs. *EMBO J.* 2019-10-18 [PMID: 31625181]

Gu S, Maurya S, Lona A et al. Ligand-Dependent Mechanisms of C-C Chemokine Receptor 5 (CCR5) Trafficking Revealed by APEX2 Proximity Labeling Proteomics *bioRxiv* : the preprint server for biology 2023-11-03 [PMID: 37961097] (ICC/IF, Human)

More publications at <http://www.novusbio.com/NBP1-49643>



## Procedures

### Immunohistochemistry-Paraffin Embedded Sections protocol specific for TGOLN2 Antibody (NBP1-49643)

#### 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.

##### Staining:

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

### Immunocytochemistry/Immunofluorescence protocol for TGN46 Antibody (NBP1-49643)

#### Immunocytochemistry Protocol

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.



### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NBP1-49643**

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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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