Product Datasheet

Prox1 Antibody (5G10) - BSA Free
NBP1-30045

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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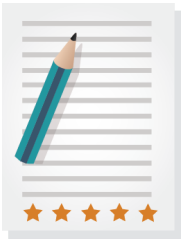
Publications: 6

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP1-30045

Updated 9/9/2025 v.20.1



NBP1-30045

Prox1 Antibody (5G10) - BSA Free

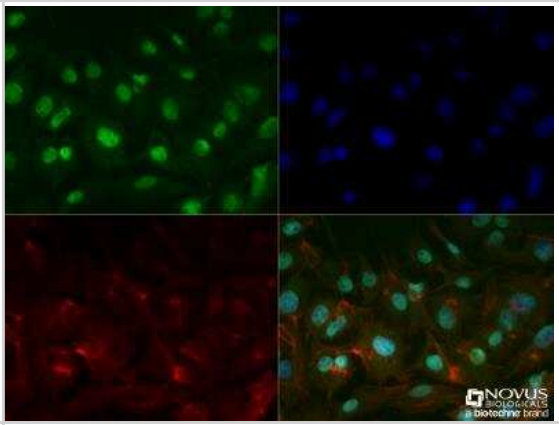
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	5G10
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Mouse Prox1 Antibody (5G10) - BSA Free (NBP1-30045) is a monoclonal antibody validated for use in IHC, ELISA, Flow and ICC/IF. Anti-Prox1 Antibody: Cited in 6 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	5629
Gene Symbol	PROX1
Species	Human, Mouse, Rat, Chicken
Reactivity Notes	It is expected to work with all vertebrates including mammals, birds and fish. Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Additional Mouse on Mouse blocking steps may be required for IHC and ICC experiments. Please contact Technical Support for more information.
Immunogen	The highly conserved Homeo and Prospero domain of human Prox1. [UniProt# Q92786]

Product Application Details	
Applications	Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, CyTOF-ready, Immunohistochemistry Whole-Mount
Recommended Dilutions	Flow Cytometry 1 - 2 ug/ml, ELISA, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:200, Immunohistochemistry-Paraffin 1:100-1:1000, Immunohistochemistry Whole-Mount reported in scientific literature (PMID 24654984), CyTOF-ready
Application Notes	This antibody is CyTOF ready.

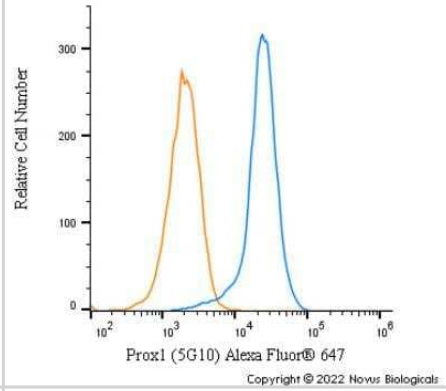
Images

Immunocytochemistry/Immunofluorescence: Prox1 Antibody (5G10) [NBP1-30045] - HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-PROX1 (5G10) NBP1-30045 at a 1:200 dilution overnight at 4C and detected with and anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was counterstained with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

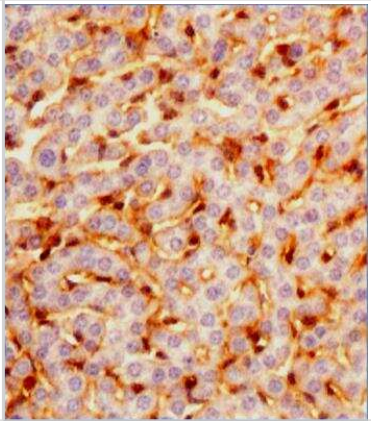


Immunohistochemistry: Prox1 Antibody (5G10) [NBP1-30045] - Rat dentate gyrus showing specific immunolabeling of the prox1 protein. Photo courtesy of Justin Kievits and Teresa Milner, Weill Cornell Medical College.

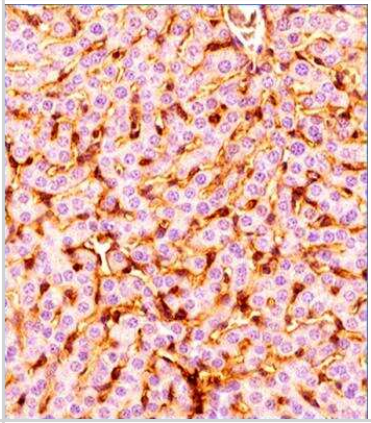
Flow Cytometry: Prox1 Antibody (5G10) [NBP1-30045] - An intracellular stain was performed on HepG2 cells with Prox1 [5G10] Antibody NBP1-30045AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



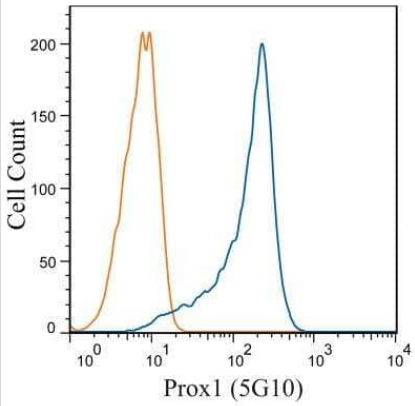
Immunohistochemistry-Paraffin: Prox1 Antibody (5G10) [NBP1-30045] - IHC analysis of a formalin fixed paraffin embedded tissue section of Mouse liver using Lot UD1009o of PROX1 antibody clone 5G10 at 1:100 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody generated nice nuclear-cytoplasmic staining in sinusoidal endothelial cells as well as in Kupffer cells.



Immunohistochemistry-Paraffin: Prox1 Antibody (5G10) [NBP1-30045] - IHC analysis of a formalin fixed paraffin embedded tissue section of Mouse liver using Lot A of PROX1 antibody clone 5G10 at 1:100 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody generated nice nuclear-cytoplasmic staining in sinusoidal endothelial cells as well as in Kupffer cells.



Flow Cytometry: Prox1 Antibody (5G10) [NBP1-30045] - HepG2 cells were stained with Prox1 (5G10) NBP1-30045 (blue) and a matched isotype control NBP2-27287 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Dylight488-conjugated anti-mouse secondary antibody.



Prox1 (5G10) was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rat anti- Prox1 (5G10) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP1-30045AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.

Publications

Iskusnykh IY, Fattakhov N, Li Y et al. Lmx1a is a master regulator of the cortical hem Elife 2023-09-19 [PMID: 37725078] (Immunohistochemistry Whole-Mount, Rat)

Rütsche D, Nanni M, Rüdisser S et Al. Enzymatically Crosslinked Collagen as a Versatile Matrix for In Vitro and In Vivo Co-Engineering of Blood and Lymphatic Vasculature Adv Mater 2023-04-21 [PMID: 36724374]

Wang T, Wang Z, de Fabritus L Et al. 1-deoxysphingolipids bind to COUP-TF to modulate lymphatic and cardiac cell development Developmental cell 2021-11-22 [PMID: 34762852]

Taira Y, Ikuta Y, Inamori S et al. The formation of multiple pituitary pouches from the oral ectoderm causes ectopic lens development in hedgehog signaling-defective avian embryos Dev. Dyn. 2020-07-07 [PMID: 32633438]

Baptista AP, Gola A, Huang Y et al. Human organotypic lymphatic vessel model elucidates microenvironment-dependent signaling and barrier function Biomaterials 2019-05-25 [PMID: 31154151] (FLOW, Human)

Sweat RS, Sloas DC, Murfee WL. VEGF-C Induces Lymphangiogenesis and Angiogenesis in the Rat Mesentery Culture Model. Microcirculation 2014-03-22 [PMID: 24654984] (IHC-WhMt, Rat)

Procedures

Flow (Intracellular) Protocol for Prox1 Antibody (NBP1-30045)
Protocol for Flow Cytometry Intracellular Staining (general dilution 1-5 ug/ml)
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 1 mL 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:
Optional: Perform cell surface staining as described in the previous section.
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 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 uL/ 1 x 106 cells of primary antibody or 1-3 uL/ 1 x 106 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 uL per sample and proceed with flow analysis.

Immunocytochemistry/ Immunofluorescence Protocol for Prox1 Antibody (NBP1-30045)
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.

Immunohistochemistry-Paraffin Protocol for Prox1 Antibody (NBP1-30045)
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 at all times).

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 NBP1-30045

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NBP1-30045R	Prox1 Antibody (5G10) [DyLight 550]

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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