Product Datasheet

PAR1/Thrombin Receptor Antibody (N2-11) - BSA Free NBP1-71770

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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NBP1-71770

PAR1/Thrombin Receptor Antibody (N2-11) - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	N2-11
Preservative	0.05% Sodium Azide
Isotype	lgG1
Purity	Protein G purified
Buffer	Tris-Glycine and 0.15M NaCl
Target Molecular Weight	52 kDa
Product Description	
Host	Mouse
Gene ID	2149
Gene Symbol	F2R
Species	Human, Mouse
Immunogen	The oligopeptide CNATLDPRSFLL from human Thrombin Receptor. [Swiss- Prot# P25116]
Product Application Details	
Applications	Western Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry- Paraffin, Immunofluorescence
Recommended Dilutions	Western Blot 1:1000, ELISA 1:100 - 1:2000, Immunohistochemistry 1:100. Use reported in scientific literature (PMID 35055169), Immunocytochemistry/ Immunofluorescence 1:50, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen reported in scientific literature (PMID 31885791), Immunofluorescence

Images

Immunohistochemistry: PAR1/Thrombin Receptor Antibody (N2-11) [NBP1-71770] - PAR1 is expressed by neural stem cells in the subventricular zone (SVZ) of the adult mouse brain. Photomicrographs show immunofluorescence double-labeling for PAR1 with Sox2-positive multipotent neural stem cells (NSCs) within the lateral wall of the lateral ventricle (LV). RNAscope was used to identify cells expressing both PAR1 and Sox2. RNA in NSCs of the adult SVZ. Arrow indicates an example of a double-labeled cell in each case, with arrowhead indicating a singly labeled cell (Scale bar = 10 um). Boxed area in B is also shown at higher magnification to visualize double-labeled cells. Image collected and cropped by Citeab from the following publication (The Thrombin Receptor Restricts Subventricular Zone Neural Stem Cell Expansion and Differentiation. Sci Rep (2018) licensed under a CC-BY license.









Immunohistochemistry: PAR1/Thrombin Receptor Antibody (N2-11) [NBP1-71770] - Analysis of Thrombin Receptor on mouse skin using NBP1-71770.

С Nestin PAR1 PAR1 is expressed by neural stem cells in the sub-ventricular zone DAPI Merge (SVZ) of the adult mouse brain. Photomicrographs show immunofluorescence double-labeling for PAR1 with Sox2-positive (A), or PAR1 with Nestin-positive (C) multipotent neural stem cells (NSCs) within the lateral wall of the lateral ventricle (LV). RNAscope was used to identify cells expressing both PAR1 & Sox2 (B), or Nestin (D) RNA in LV NSCs of the adult SVZ. Arrow indicates an example of a double-labeled cell in each case, with arrowhead indicating a singly labeled cell (Scale bar = 10 μ m). Boxed area in B & D is also shown at higher magnification to visualize double-labeled cells. (E) Histogram shows expression of PAR1 RNA was high in NSCs grown as neurospheres (NS), or when plated on poly-L-lysine coated coverslips as monolayers in stem cell media containing EGF & bFGF. PAR1 RNA expression by NSC monolayers decreased by 87% when EGF & bFGF were removed from the media for 7 DIV promoting stem cell differentiation. (F) Withdrawal of EGF & bFGF to induce NSC monolayer differentiation resulted in a parallel decrease in Nestin RNA expression. (**P < 0.01, ***P < 0.001 Students t-test). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29921916), licensed under a CC-BY license. Not internally tested by Novus Biologicals. А Immunocytochemistry/ Immunofluorescence: PAR1/Thrombin Receptor Sox2 PAR1 DAPI Merge Antibody (N2-11) [NBP1-71770] - PAR1 is expressed by neural stem cells in the sub-ventricular zone (SVZ) of the adult mouse brain. Photomicrographs show immunofluorescence double-labeling for PAR1 with Sox2-positive (A), or PAR1 with Nestin-positive (C) multipotent LV neural stem cells (NSCs) within the lateral wall of the lateral ventricle (LV). RNAscope was used to identify cells expressing both PAR1 & Sox2 (B), or Nestin (D) RNA in NSCs of the adult SVZ. Arrow indicates an example of a double-labeled cell in each case, with arrowhead indicating a singly labeled cell (Scale bar = $10 \mu m$). Boxed area in B & D is also shown at higher magnification to visualize double-labeled cells. (E) Histogram shows expression of PAR1 RNA was high in NSCs grown as neurospheres (NS), or when plated on poly-L-lysine coated coverslips as monolayers in stem cell media containing EGF & bFGF. PAR1 RNA expression by NSC monolayers decreased by 87% when EGF & bFGF were removed from the media for 7 DIV promoting stem cell differentiation. (F) Withdrawal of EGF & bFGF to induce NSC monolayer differentiation resulted in a parallel decrease in Nestin RNA expression. (**P < 0.01, ***P < 0.001 Students t-test). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29921916), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

YS Hwang, HJ Cho, ES Park, J Lim, HR Yoon, JT Kim, SR Yoon, H Jung, YK Choe, YH Kim, CH Lee, YT Kwon, BY Kim, HG Lee KLK6/PAR1 Axis Promotes Tumor Growth and Metastasis by Regulating Cross-Talk between Tumor Cells and Macrophages Cells, 2022-12-16;11(24):. 2022-12-16 [PMID: 36552865]

Hayashi Y, Shimizu I, Yoshida Y et al. Coagulation factors promote brown adipose tissue dysfunction and abnormal systemic metabolism in obesity iScience 2022-07-15 [PMID: 35754738] (IHC-P, Mouse)

Goldberg Z, Sher I, Qassim L et al. Intrinsic Expression of Coagulation Factors and Protease Activated Receptor 1 (PAR1) in Photoreceptors and Inner Retinal Layers International journal of molecular sciences 2022-01-17 [PMID: 35055169] (WB, IF, Mouse)

Chen R, Cao X, et al. Dabigatran Suppresses PAR-1/SphK/S1P Activation of Astrocytes in Experimental Autoimmune Encephalomyelitis Model. Front Mol Neurosci 2020-07-23 [PMID: 32694981]

Nishimura F, Mogami H, Moriuchi K et al. Mechanisms of thrombin-Induced myometrial contractions: Potential targets of progesterone PLoS ONE 2020-05-04 [PMID: 32365105] (IHC-P, Human)

Kim MJ, Park KH, Lee JY et al. Weisheng-Tang Ameliorates Acute Ischemic Brain Damage in Mice by Maintaining Blood-Brain Barrier Integrity Oxid Med Cell Longev 2019-12-03 [PMID: 31885791] (IHC-Fr, Mouse)

Komori M, Ago T, Wakisaka Y, et al. Early initiation of a factor Xa inhibitor can attenuate tissue repair and neurorestoration after middle cerebral artery occlusion Brain Res. 2019-05-16 [PMID: 31103522] (IF/IHC, Mouse)

Choi CI, Yoon H, Drucker KL et al. The Thrombin Receptor Restricts Subventricular Zone Neural Stem Cell Expansion and Differentiation Sci Rep 2018-06-19 [PMID: 29921916] (IF/IHC, ICC/IF, Mouse)

Kim HN, Kim YR, Ahn SM et al. Protease activated receptor-1 antagonist ameliorates the clinical symptoms of experimental autoimmune encephalomyelitis via inhibiting breakdown of blood brain barrier. J. Neurochem. 2015-08-18 [PMID: 26285165] (WB, Mouse)

Gonda K, Watanabe TM, Ohuchi N, Higuchi H. In vivo nano-imaging of membrane dynamics in metastatic tumor cells using quantum dots. J Biol Chem;285(4):2750-7. 2010-01-22 [PMID: 19917603] (ICC/IF, Mouse)



Procedures

Western Blot Protocol Specific for NBP1-71770 [Thrombin Receptor Antibody] (NBP1-71770)

PAR1/Thrombin Receptor Antibody (N2-11): Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.

2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.

3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.

4. Rinse the blot.

5. Block the membrane using standard blocking buffer for at least 1 hour.

6. Wash the membrane in wash buffer three times for 10 minutes each.

7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.

8. Wash the membrane in wash buffer three times for 10 minutes each.

9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.

10. Wash the blot in wash buffer 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.

*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin protocol for Thrombin Receptor Antibody (NBP1-71770) PAR1/Thrombin Receptor Antibody (N2-11):

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.

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14. Dehydrate sections.

15. Mount coverslips.



Immunocytochemistry/Immunofluorescence Protocol for Thrombin Receptor Antibody (NBP1-71770)

PAR1/Thrombin Receptor Antibody (N2-11): Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.

2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.

3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.

4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.

5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.

6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.

7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.

8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.

9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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Products Related to NBP1-71770

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NBP1-71770B	PAR1/Thrombin Receptor Antibody (N2-11) [Biotin]

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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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