Product Datasheet

TRPM8 Antibody - BSA Free
NBP1-97311

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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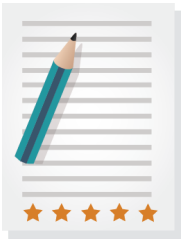
Updated 10/23/2024 v.20.1



NBP1-97311

TRPM8 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	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS (pH 7.4)

Product Description

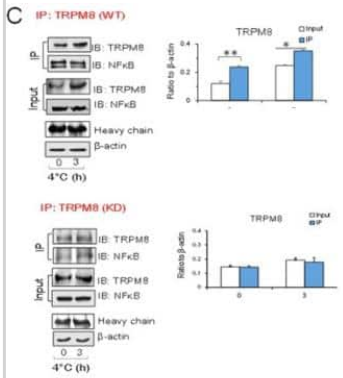
Host	Rabbit
Gene ID	79054
Gene Symbol	TRPM8
Species	Human, Mouse, Rat
Immunogen	A synthetic peptide made to an internal portion of the human TRPM8 protein (between residues 250-300) [UniProt# Q7Z2W7]

Product Application Details

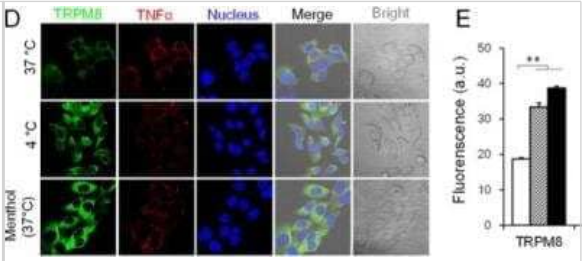
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:300, Knockdown Validated
Application Notes	In Western Blot a band is seen at ~127 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

Images

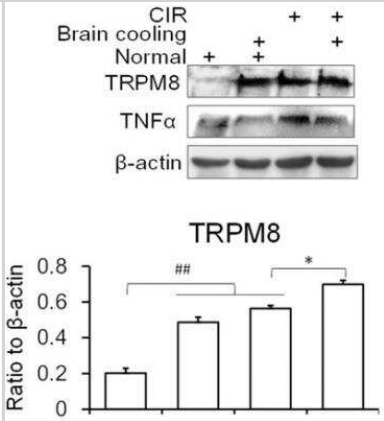
Knockdown Validated: TRPM8 Antibody [NBP1-97311] - The results of co-immunoprecipitation (CoIP) confirmed interaction between NFkB and TRPM8. CoIPs were also performed with TRPM8 antibodies. Western blot analysis was carried out by TRPM8 and NFkB. Data are shown as the mean +/- S.D. from three experiments. *P < 0.05; **P < 0.01. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155) licensed under a CC-BY license.



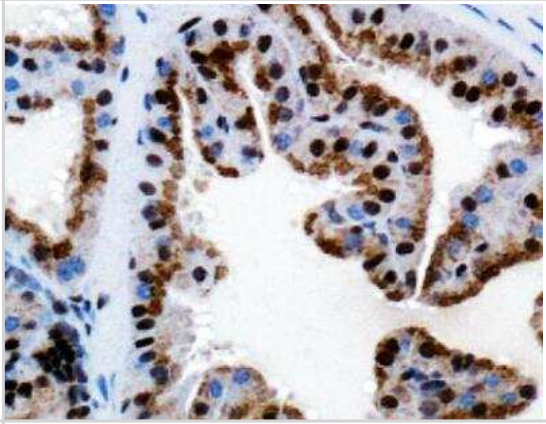
Immunocytochemistry/Immunofluorescence: TRPM8 Antibody [NBP1-97311] - Co-localization of TRPM8 and TNFa in the cytoplasm (cold condition and 500 nM menthol). Data are shown as the mean +/- S.D. from three experiments. **P < 0.01.Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155) licensed under a CC-BY license.



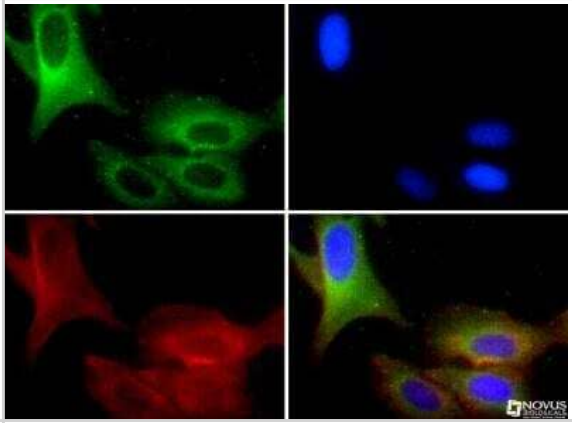
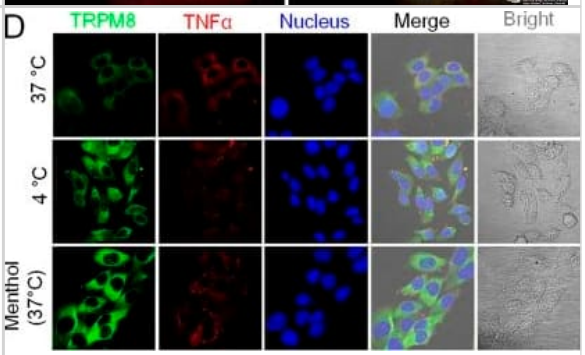
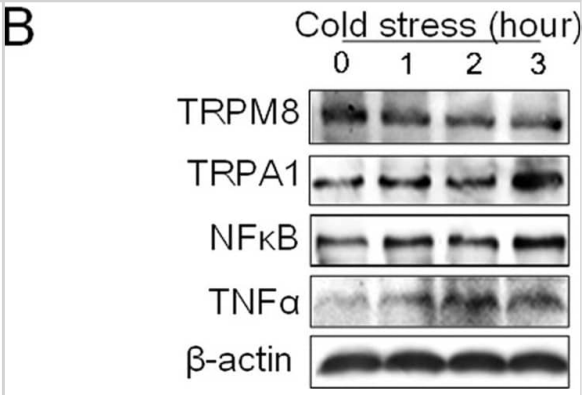
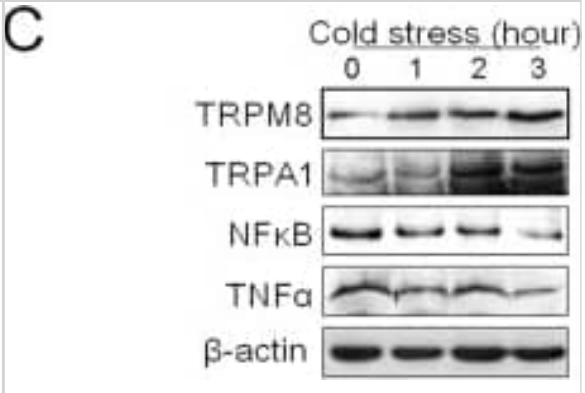
Western Blot: TRPM8 Antibody [NBP1-97311] - Brain cooling means putting the anesthetized mouse brain on the artificial ice. Normal means the room temperature (25C). Data are shown as the mean +/- S.D. from five mice in each group. #P < 0.05; ##P < 0.01, v.s. the normal control. *P < 0.05; v.s. the CIR. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155) licensed under a CC-BY license.

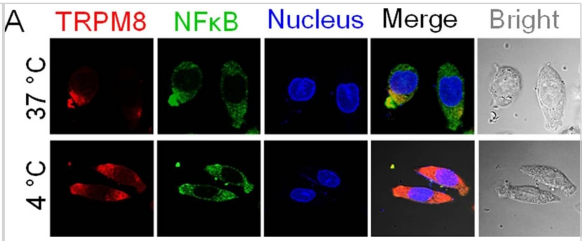
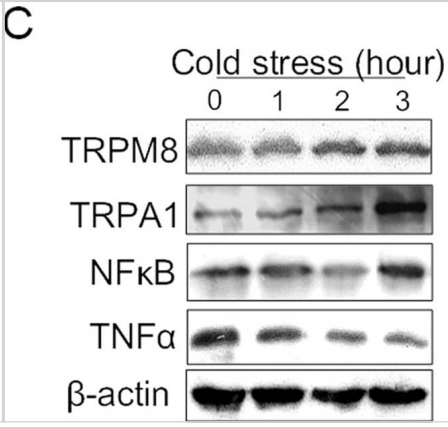


Immunohistochemistry: TRPM8 Antibody [NBP1-97311] - Analysis of TRPM8 in mouse prostate using DAB with hematoxylin counterstain.



Western Blot: TRPM8 Antibody [NBP1-97311] - Analysis of TRPM8 in PC12 cell lysate.

Immunocytochemistry/Immunofluorescence: TRPM8 Antibody [NBP1-97311] - TRPM8 antibody was tested in U2OS cells with FITC (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).	
Immunocytochemistry/ Immunofluorescence: TRPM8 Antibody - BSA Free [NBP1-97311] - Confocal imaging of TRPM8, NFκB & TNFα expression in PC12 cells.(A) The expression of TRPM8, NFκB & TNFα in wild type cells & Trpm8 knockdown cells at 37 °C & 4 °C. KD signifies Trpm8 knockdown cells. (B) In wild type cells, TRPM8 was upregulated & NFκB & TNFα were downregulated under cold conditions. (C) In KD cells, TRPM8 showed weak expression & NFκB & TNFα expression levels were increased. (D) Co-localization of TRPM8 & TNFα in the cytoplasm (cold condition & 500 nM menthol). Data are shown as the mean ± S.D. from three experiments. *P < 0.05; **P < 0.01. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: TRPM8 Antibody - BSA Free [NBP1-97311] - Expression of TRPM8, TRPA1, NFκB & TNFα in PC12 cells with Trpm8 knockdown under cold conditions (4 °C).(A) Construction of a Trpm8 (Dylight 649) knockdown stable cell line. WT represents wild type cells. KD signifies the Trpm8 knockdown cells. (B,C) Protein expression levels of TRPM8, TRPA1, NFκB & TNFα. NS: no significance. Data are shown as the mean ± S.D. from three experiments. #P < 0.05; ##P < 0.01, v.s. the control (zero time). Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: TRPM8 Antibody - BSA Free [NBP1-97311] - Alteration of core temperature & the expression levels of TRPM8, TRPA1, NFκB & TNFα in mouse brains under cold conditions.(A) Core body temperature under cold conditions (4 °C). (B) The mRNA expression levels of TRPM8, TRPA1, NFκB & TNFα. (C,D) The protein expression levels of TRPM8, TRPA1, NFκB & TNFα. Data are shown as the mean ± S.D. from 12 mice in each group. ##v.s. the control (zero hour), P < 0.01; #P < 0.05. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Immunocytochemistry/ Immunofluorescence: TRPM8 Antibody - BSA Free [NBP1-97311] - Co-localization of TRPM8 & NFκB in PC12 cells under cold conditions.(A) Immunofluorescence assay image of TRPM8 & NFκB in the cytoplasm. WT represents wild type cells. KD represents Trpm8 knockdown cells. (B) The results of co-immunoprecipitation (CoIP) of endogenous TRPM8 & NFκB using NFκB antibodies. Western blot analysis was carried out to detect TRPM8 & NFκB. (C) Reverse CoIP confirmed interaction between NFκB & TRPM8. CoIPs were also performed with TRPM8 antibodies. Western blot analysis was carried out by TRPM8 & NFκB. Data are shown as the mean ± S.D. from three experiments. *P < 0.05; **P < 0.01. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: TRPM8 Antibody - BSA Free [NBP1-97311] - Expression of TRPM8, TRPA1, NFκB & TNFα in PC12 cells under cold conditions. (A) Intracellular Ca2+ in the cells under cold conditions (4 °C). The Ca2+ concentration in the cytoplasm at 4 °C is higher than that at 37 °C. (B) The mRNA expression levels of TRPM8, TRPA1, NFκB & TNFα. (C,D) The protein expression levels of TRPM8, TRPA1, NFκB & TNFα. Data are shown as the mean ± S.D. from three experiments. #P < 0.05; ##P < 0.01, v.s. the control (zero time). Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep45155), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Di Sarno, V;Giovannelli, P;Medina-Peris, A;Ciaglia, T;Di Donato, M;Musella, S;Lauro, G;Vestuto, V;Smaldone, G;Di Matteo, F;Bifulco, G;Castoria, G;Migliaccio, A;Fernandez-Carvajal, A;Campiglia, P;Gomez-Monterrey, I;Ostacolo, C;Bertamino, A; New TRPM8 Blockers Exert Anticancer Activity Over Castration-Resistant Prostate Cancer Models SSRN Electronic Journal [PMID: 35598411]

Zhao M, Chen Z, Liu L et al. Functional Expression of Transient Receptor Potential and Piezo1 Channels in Cultured Interstitial Cells of Human-Bladder Lamina Propria Frontiers in physiology 2022-01-06 [PMID: 35069237] (IF/IHC, Human)

Di Donato M, Ostacolo C, Giovannelli P et al. Therapeutic potential of TRPM8 antagonists in prostate cancer Scientific reports 2021-12-01 [PMID: 34853378] (WB, Human)

Ezzatpanah S, Eriksen MB, Gjestvang Moe AM, Haugen F Diminished Cold Avoidance Behaviours after Chronic Cold Exposure - Potential Involvement of TRPM8 Neuroscience 2021-06-15 [PMID: 34139303] (WB, Mouse)

Paris AJ, Hayer KE, Oved JH et al. Increased Transient Receptor Potential Melastatin 8 Expression in the development of bladder pain in patients with Interstitial cystitis/Bladder Pain Syndrome Nat Cell Biol 2020-10-10 [PMID: 32989251] (IF/IHC, WB, Mouse, Human)

Taylor DJR, Hamid SM, Andres AM et al. Antiviral Effects of Menthol on Coxsackievirus B Viruses 2020-03-28 [PMID: 32231022] (WB, Human)

Brandolini L, Castelli V, Aramini A et al. DF2726A, a new IL-8 signalling inhibitor, is able to counteract chemotherapy-induced neuropathic pain Sci Rep 2019-08-13 [PMID: 31409858] (WB, Rat)

Wang XP, Yu X, Yan XJ et al. TRPM8 in the negative regulation of TNFa expression during cold stress. Sci Rep. 2017-03-23 [PMID: 28332601] (IF/IHC, Rat)

Procedures

Serum protocol for TRPM8 Antibody (NBP1-97311)
TRPM8 Antibody:
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 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 4 C.
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 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 NBP1-97311

NBP1-97311PEP	TRPM8 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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