# **Product Datasheet**

# PIEZO2 Antibody - BSA Free NBP1-78624

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 10/29/2024 v.20.1

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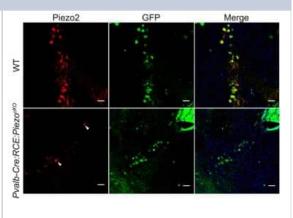
### NBP1-78624

PIEZO2 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	
Host	Rabbit
Gene ID	63895
Gene Symbol	PIEZO2
Species	Human, Mouse, Rat, Guinea Pig
Reactivity Notes	Use in Rat reported in scientific literature (PMID:34335288) Guinea Pig reactivity reported in scientific literature (PMID: 30324494).
Immunogen	A synthetic peptide made to an internal portion of the human PIEZO2 protein (between residues 1600-1650) [UniProt# Q9H5I5]
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml. Use reported in scientific literature (PMID 31791130), Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1 - 2 ug/ml, Immunohistochemistry-Paraffin 1:200
Application Notes	In ICC/IF, membrane staining was observed in A431 cells. In paraffin sections, membrane and cytoplasmic staining is seen in mouse epidermis tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

#### Images

PIEZO2 expression and mechanical characterization of MTN neurons in Piezo2cKO mice. Coronal brainstem sections of MTN neurons with DAPI stained nuclei (blue) that were immunolabeled for PIEZO2 (red, left) or GFP (green, middle: parvalbumin-positive cell) and the merged image of both. In WT littermates mice PIEZO2 was detected in the 23 GFP+ MTM neurons (top) and PIEZO2 was detected in 7 MTM neurons of the 20 that expressed GFP in Pvalb-Cre:RCE:Piezo2cKO mice (bottom; arrowhead, PIEZO2+ neuron). Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep25923) licensed under a CC-BY license.



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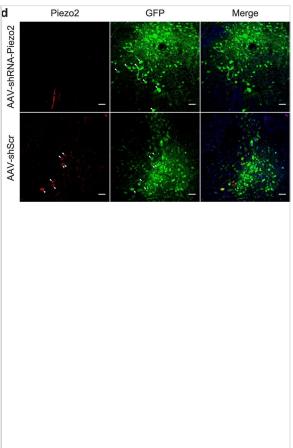
Analysis of PIEZO2 in dorsal root ganglion of embryonic day (E) 13.5 mouse. PIEZO2 in red, DAPI in blue. Antigen retrieval with 1x DAKO antigen retrieval solution, by heating in a microwave oven, for total time of 6 minutes 10 seconds. Washing buffer 1 x PBS with 0.1% Triton X-100. Blocking in 5% donkey serum (Jackson lab) in washing buffer. Primary antibody diluted 1:1000, incubation 18 hours in room temperature. Secondary antibody donkey anti-rabbit Cy3, 1:400, 3 hours in room temperature. Image from verified customer review. Immunocytochemistry/Immunofluorescence: PIEZO2 Antibody [NBP1-78624] - PIEZO2 antibody was tested at 1:50 in A431 cells with FITC (green) .Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red). Analysis of PIEZ02 in mouse epidermis using DAB with hematoxylin counterstain. Analysis of a FFPE tissue section of human breast using 1:200 dilution of PIEZO2 antibody. The staining was developed using HRP labeled antirabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.

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Immunocytochemistry/ Immunofluorescence: PIEZO2 Antibody - BSA Free [NBP1-78624] - Suppression of MA in MTN neurons from adult C57BL/6J mice by silencing Piezo2.(a) Schematic representation of the unilateral adeno-associated viral (AAV) injection site targeting MTN neurons. (b) Fluorescence images showing the expression of GFPtagged MTN neurons in brain slices following AAV delivery. (b') Higher magnification of the boxed area in (b). (c) The AAV-Piezo2-sh-1 construct. (d) Brainstem coronal sections of C57BL/6J mice infected with the AVV-shRNA against Piezo2 (middle) & the scrambled AVV (lower), showing MTN neurons immunolabeled for Piezo2 (left, red) or GFP (middle, green), & the merged image of both, with the nuclei stained with DAPI (blue). Arrowheads indicate representative MTN neurons. Scale bar 50 µm. (e) Traces of MA currents evoked by mechanical indentation in MTN neurons infected with AAV-Piezo2-sh-1 or with a scrambled AVV (f). (g) Trace from a MTN neuron infected with AAV-Piezo2-sh-1 that responded with RA mechanical activated currents ( $\tau = 6.2$  ms). The inset shows a higher magnification of the current trace corresponding to a 7 µm displacement. The black upper trace shows the 250 ms mechanical stimulus steps in 1 µm increments applied every 10 s, the breaks in the trace corresponding to equivalent breaks in the recording. (h) Histogram summarizing the mean amplitude of the RA currents in MTN neurons from WT mice (n = 69) & those infected with AAV-Piezo2-sh-1 (n = 20) or AAV-shScr (n = 8). The data are expressed as the means  $\pm$  s.e.m.: \*p < 0.05 (Student's t-test). Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep25923), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





#### **Publications**

Tiwari N, Smith C, Sharma D et al. Plp1-expressing perineuronal DRG cells facilitate colonic and somatic chronic mechanical pain involving Piezo2 upregulation in DRG neurons. Cell reports 2024-05-31 [PMID: 38743566]

Xingyang Wan, Qian Zhou, Huaxian Chen, Zhen Li, Mianling Mo, Zhimin Liu, Heng Zhang, Zhuojie He, Guozhong Xiao, Yihui Zheng, Hongcheng Lin, Donglin Ren Astragaloside IV improves slow transit constipation by regulating gut microbiota and enterochromaffin cells Frontiers in Pharmacology 2023-11-21 [PMID: 38074145]

Madar J, Tiwari N, Smith C et al. Piezo2 regulates colonic mechanical sensitivity in a sex specific manner in mice Nature communications 2023-04-15 [PMID: 37061508]

Jia X, Liu X, Zhu T et al. Infiltrated Macrophages Aggravate TMJOA Chronic Pain Via Piezo2 in IB4 -TG Neurons papers.ssrn.com 2023-11-28 (IHC-Fr, ICC/IF, Rat)

Chen G, Gao X, Chen J et al. Actomyosin Activity and Piezo1 Activity Synergistically Drive Urinary System Fibroblast Activation Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2023-10-22 [PMID: 37867255] (WB, Human)

Liu L, Zhao Y, An W et al. Piezo2 Channel Upregulation is Involved in Mechanical Allodynia in CYP-Induced Cystitis Rats Molecular Neurobiology 2023-09-01 [PMID: 37227654] (Western Blot)

Michalak-Micka K, R tsche D, Mazzone L et al. Human fetal skin derived merkel cells display distinctive characteristics in vitro and in bio-engineered skin substitutes in vivo Frontiers in Bioengineering and Biotechnology 2022-09-15 [PMID: 36185452] (Immunocytochemistry/ Immunofluorescence)

Duan M, Jia Y, Huo L et al. Potentiation of PIEZO2 mechanically-activated currents in sensory neurons mediates vincristine-induced mechanical hypersensitivity Acta Pharmaceutica Sinica B 2023-08-01 [PMID: 37655331] (Block/Neutralize)

Fang J, Hou F, Wu S et al. Piezo2 downregulation via the Cre-lox system affects aqueous humor dynamics in mice Mol Vis 2021-05-20 [PMID: 34220183] (Immunohistochemistry)

Scavuzzo MA, Letai KC, Maeno-Hikichi Y et al. Enteric glial hub cells coordinate intestinal motility bioRxiv : the preprint server for biology 2023-06-09 [PMID: 37333182] (IHC, Mouse)

Duan M, Jia Y, Huo L et al. Potentiation of PIEZO2 mechanically-activated currents in sensory neurons mediates vincristine-induced mechanical hypersensitivity Acta Pharmaceutica Sinica B 2023-05-01 (Immunohistochemistry, Rat)

Gao S, Tang Y, Sun W et al. 3D-bioprinted GelMA nerve guidance conduits promoted peripheral nerve regeneration by inducing trans-differentiation of MSCs into SCLCs via PIEZO1/YAP axis Materials Today Advances 2023-03-01 (WB, Rat)

More publications at <a href="http://www.novusbio.com/NBP1-78624">http://www.novusbio.com/NBP1-78624</a>



#### **Procedures**

#### Western Blot protocol specific for PIEZO2 antibody (NBP1-78624) WB

#### 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%.

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

#### Immunohistochemistry-Paraffin protocol for PIEZO2 Antibody (NBP1-78624)

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.

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#### Immunocytochemistry/Immunofluorescence protocol for PIEZO2 Antibody (NBP1-78624)

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 NBP1-78624

NBP1-78624PEP	PIEZO2 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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