# **Product Datasheet**

# PIEZO1 Antibody - BSA Free NBP1-78537

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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### NBP1-78537

PIEZO1 Antibody - 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	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Product Description	
Host	Rabbit
Gene ID	9780
Gene Symbol	PIEZO1
Species	Human, Mouse
Immunogen	A synthetic peptide made to an internal portion of the human PIEZO1 protein (between residues 1300-1350) [UniProt Q92508]
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin

# Images

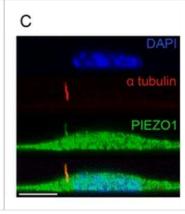
**Application Notes** 

**Recommended Dilutions** 

Immunocytochemistry/Immunofluorescence: PIEZO1 Antibody [NBP1-78537] - Localization of PIEZO1 on MLO-Y4 primary cilia and plasma membrane. Acetylated alpha-tubulin is in enriched primary cilia. Scale bars, 10 um. Image collected and cropped by CiteAb from the following publication (https://www.ciliajournal.com/content/4/1/7) licensed under a CC-BY license.

Paraffin 1:400

buffer (pH 6.0) is recommended.

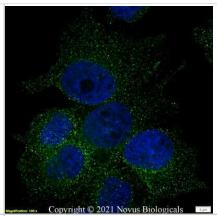


Western Blot 2 ug/mL, Simple Western 1:160, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:25, Immunohistochemistry-

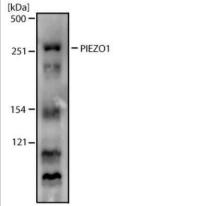
Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate



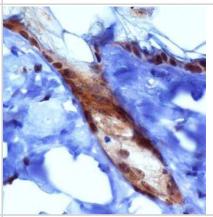
Immunocytochemistry/Immunofluorescence: PIEZO1 Antibody [NBP1-78537] - MCF7 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NBP1-78537 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



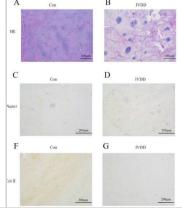
Western Blot: PIEZO1 Antibody [NBP1-78537] - Analysis of MCF7 cell lysate using PIEZ01 antibody (NBP1-78537) at 2 ug/mL.



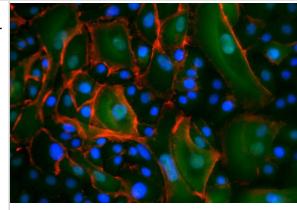
Immunohistochemistry: PIEZO1 Antibody [NBP1-78537] - Analysis of PIEZ01 in mouse epidermis using DAB with hematoxylin counterstain.



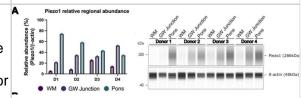
Immunohistochemistry: PIEZO1 Antibody [NBP1-78537] - Histological analysis and the expression of PIEZO1 (NBP1-78537) and collage II (NBP1-77795) in human nucleus pulposus tissues. A, B Hematoxylin and eosin (H&E) staining in human NP tissues of the control or the IVDD group (200×). C, D PIEZO1 immunostaining in human NP tissues of the control or the IVDD group (100×). E The percentage of PIEZO1 positive cells to all cells in the field (%). F, G Collagen II immunostaining in human NP tissues of the control or the IVDD group (100×). Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/35606793/) licensed under a CC-BY license.



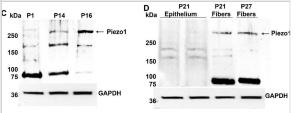
Immunocytochemistry/Immunofluorescence: PIEZO1 Antibody [NBP1-78537] - Green: 2 hours at RT with CLTC antibody diluted 1:100 in PBS-Triton 0.2%X-100/BSA 1%. Red: Phalloidin Blue: DAPI. Image from verified customer review.



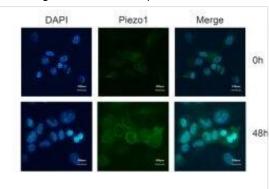
Simple Western: PIEZO1 Antibody - BSA Free [NBP1-78537] - Piezo1 expression profiling, cellular mechanotransduction, and biomechanical correlation analysis across brain regions of different donors. The relative abundance of (A) Piezo1, (B) YAP, (C) pYAP, and (D) beta-catenin in the regions of WM (magenta), GW junction (purple), and pons (green) for individual donors (D1-4). (E, top left) Spearman correlation between Piezo1 relative fluorescence units (RFU) and stiffness; GW Junction (orange) ns, WM (blue) r = -0.5341, p < 0.05, pons (green) ns. (E, top right) Correlation between Piezo1 regional RFU and spring term; GW junction r = 0.8791, and WM r = -0.5341, p < 0.05. (E, bottom left) Correlation between Piezo1 regional RFU and decay term (b); WM r = -0.6758, and pons r = 0.7692, p < 0.05. (E, bottom right) Correlation between Piezo1 regional RFU and equilibrium stress term; WM r = -0.8571, pons r = 0.7198, p < 0.05. (F) Comparison of protein expressed based on brain region for all donors, \*p < 0.05 Image collected and cropped by CiteAb from the following publication (https://molecularbrain.biomedcentral.com/articles/10.1186/s13041-023-01071-5), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



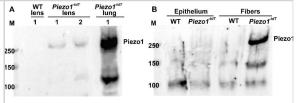
Western Blot: PIEZO1 Antibody - BSA Free [NBP1-78537] - Expression & distribution of Piezo channels in mouse lenses. (A). RT-PCR-based confirmation of Piezo1 & Piezo2 expression in P1 & P30 mouse lenses. (B). gRT-PCR analysis revealed a relatively much higher level of Piezo1 expression in the lens (P30) compared to Piezo2. (C). Total lysates (800× g supernatants; 75 µg protein) derived from the P1, P14, & P16 mouse lenses analyzed using a Piezo1 polyclonal antibody exhibited immunopositive bands with an expected molecular mass of >250 kDa & >150 kDa. There was also a prominent immunopositive band at >75 kDa in the P1 & P14 lenses, the levels of which appeared to be decreased in the P16 lenses. (D). Piezo1 immunopositive bands of >250 & >75 kDa were present predominantly in the lens fiber samples (P21 & P27) compared to the lens epithelium (P21). (E,F). Immunofluorescence analysis of Piezo1 in the P1 mouse lens (the sagittal plane of the cryosection) revealed that the protein distributes predominantly to lens fibers relative to the epithelium (boxed area in panel (E) was magnified & shown in panel (F)). (G) Shows background immunofluorescence with secondary antibody alone. GAPDH: Loading control; Epi: Epithelium; Bars: Image magnification. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35563101), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemistry: PIEZO1 Antibody - BSA Free [NBP1-78537] -Piezo1 expression & cell function of human nucleus pulposus cells under mechanical stress. A NP cells received various mechanical stress treatment. Cell viability was measured by MTT assay. B Gene expression of Piezo1 was measured by real-time PCR. C. D Western blot analysis of Piezo1's protein level. Total β-actin served as loading controls. E, F Immunofluorescence staining analysis of Piezo1 expression. Relative fluorescent levels of Piezo1 were measured by Image J software. G Concentrations of pro-inflammatory cytokines TNF-α, IL-1β, & IL-6 in supernatants were measured by ELISA. H Mitochondrial membrane potential was measured by JC-1 probe & flow cytometer. I OCR of cells were measured by Seahorse XFe96 Extracellular Flux Analyzer at basal conditions & with serial administration of oligomycin, FCCP & rotenone. J-L Gene & protein expressions of P53 & P16. Total β-actin served as loading controls. \*p < 0.05. All experiments were repeated at least three times Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35606793), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: PIEZO1 Antibody - BSA Free [NBP1-78537] - The piezo1tdT mouse confirms the expression & distribution of Piezo1 in lens fibers. (A). The Piezo1-tdT mouse model expressing a fusion protein of Piezo1 & tdTomato (Piezo1-tdT) was used to determine the distribution pattern of Piezo1 in the mouse lens. Similar to what was found in the wild-type lenses (Figure 3), Piezo1-tdT exhibiting the expected molecular mass of >250 kDa was detected only in P30 lens homogenates derived from the Piezo1-tdT mice, but not in the wild-type lens. The positive control (lung tissue lysate from the Piezo1-tdT mice) also showed a robust expression of Piezo1-tdT. Lanes 1 & 2 represent two different loads of the total protein (75 & 150 µg, respectively). (B) The Piezo1-tdT fusion protein was detected predominantly in fiber cell lysates compared to lens epithelial lysates. (C). Immunofluorescence analysis revealed the Piezo1 -tdT fusion protein distributing to lens fibers with localization to both the short & long arms of the hexagonal lens fibers (Left & middle panels are with low & high magnification, respectively). The right panel shows a second antibody (Alexa Flour 488) background control fluorescence staining in the Piezo1-tdT mouse lens section. Bars: Image magnification. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35563101), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



#### **Publications**

Johnson RT, Solanki R, Wostear F, Ahmed S et Al. Piezo1-mediated regulation of smooth muscle cell volume in response to enhanced extracellular matrix rigidity Br J Pharmacol 2023-12-03 [PMID: 38044463]

Johnson RT, Wostear F, Solanki R et Al. A microtubule stability switch alters isolated vascular smooth muscle Ca2+ flux in response to matrix rigidity J Cell Sci 2024-11-12 [PMID: 39301761]

Walker M, Pringle EW, Ciccone G et al. Mind the Viscous Modulus: The Mechanotransductive Response to the Viscous Nature of Isoelastic Matrices Regulates Stem Cell Chondrogenesis Advanced Healthcare Materials 2023-12-12 [PMID: 38014647]

S Shi, XJ Kang, Z Zhou, ZM He, S Zheng, SS He Excessive mechanical stress-induced intervertebral disc degeneration is related to Piezo1 overexpression triggering the imbalance of autophagy/apoptosis in human nucleus pulpous Arthritis Research & Therapy, 2022-05-23;24(1):119. 2022-05-23 [PMID: 35606793]

Zhu Z, Chen X, Chen S et al. Examination of the mechanism of Piezo ion channel in 5-HT synthesis in the enterochromaffin cell and its association with gut motility Frontiers in endocrinology 2023-11-02 [PMID: 38027192]

Raha A, Wu Y, Zhong L et al. Exploring Piezo1, Piezo2, and TMEM150C in Human Brain Tissues and Their Correlation with Brain Biomechanical Characteristics Research Square 2023-10-09 [PMID: 38124148] (Immunohistochemistry, Simple Western, Human)

Matsunaga M, Kimura M, Ouchi T et al. Mechanical Stimulation-Induced Calcium Signaling by Piezo1 Channel Activation in Human Odontoblast Reduces Dentin Mineralization Frontiers in Physiology 2021-08-24 [PMID: 34504437]

Wen D, Gao Y, Liu Y et al. Matrix stiffness-induced ?-tubulin acetylation is required for skin fibrosis formation through activation of Yes-associated protein MedComm (2020) 2023-08-01 [PMID: 37457658] (In vivo assay)

Vasileva V, Morachevskaya E, Sudarikova A et al. Selective Chemical Activation of Piezo1 in Leukemia Cell Membrane: Single Channel Analysis International Journal of Molecular Sciences 2021-07-22 [PMID: 34360605]

Zhu B, Qian W, Han C et al. Piezo 1 activation facilitates cholangiocarcinoma metastasis via Hippo/YAP signaling axis Molecular Therapy - Nucleic Acids 2021-06-04 [PMID: 33767919] (Block/Neutralize)

Zhang F, He X, Dong K et al. Combination therapy with ultrasound and 2D nanomaterials promotes recovery after spinal cord injury via Piezo1 downregulation Journal of nanobiotechnology 2023-03-15 [PMID: 36922816] (ICC/IF, IHC-Fr, Mouse)

#### Details:

Dilution used in ICC/IF and IHC-Fr 1:200

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] (IHC-Fr, Mouse)

More publications at <a href="http://www.novusbio.com/NBP1-78537">http://www.novusbio.com/NBP1-78537</a>



### **Procedures**

### Western Blot protocol for PIEZO1 Antibody (NBP1-78537)

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 PIEZO1 Antibody (NBP1-78537)

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



## Immunocytochemistry/ Immunofluorescence Protocol for PIEZO1 Antibody (NBP1-78537)

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 NBP1-78537**

NBP1-78537PEP PIEZO1 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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