NEUROMICS

285-304

ASIC3 [DRASIC] Data Sheet

Catalog Number:	GP14105	**Host:**	Guinea Pig
Product Type:	Whole Serum	**Species Reactivity:**	Rat
Immunogen Sequence:	(C)TASLDPDDFDPEPSDPLGSP Corresponding to residues 285-304 of the extracellular domain rat ASIC3 [DRASIC].	**Format:**	Liquid in phosphate buffered saline (pH 7.4) with 1% bovine serum albumin and 0.05% sodium azide.
Applications:	Immunohistochemistry 1:10-1:100 Immunocytochemistry 1:100		

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Maintain at +2-8°C for 3 months or at -20°C for longer periods. Stable for 1 year. *Avoid repeated freeze-thaw cycles.*

References:

Kiyomi Hori, Noriyuki Ozaki, Shigeyuki Suzuki, Yasuo Sugiura. Upregulations of P2X3 and ASIC3 involve in hyperalgesia induced by cisplatin administration in rats. PAIN 149 (2010) 393–405.

Yoshiyasu Uchiyama, Chin-Chang Cheng, Keith G Danielson, Joji Mochida, Todd J Albert, Irving M Shapiro and and Makarand V Risbud. Expression of Acid-Sensing Ion Channel 3 (ASIC3) in Nucleus. Pulposus Cells of the Intervertebral Disc Is Regulated by p75NTR and ERK Signaling. JOURNAL OF BONE AND MINERAL RESEARCH. Volume 22, Number 12, 2007 Published online on August 13, 2007; doi: 10.1359/JBMR.070805.© 2007 American Society for Bone and Mineral Research .

R. Ambalavanara, C. Yallampallib, U. Yallampallib and D. Dessema. Injection of adjuvant but not acidic saline into craniofacial muscle evokes nociceptive behaviors and neuropeptide expression. doi:10.1016/j.neuroscience.2007.07.058.

T. Fukuda, H. Ichikawa, R. Terayama, T. Yamaai, T. Kuboki and T. Sugimoto. ASIC3-immunoreactive neurons in the rat vagal and glossopharyngeal sensory ganglia. doi:10.1016/j.brainres.2006.01.039

Application Notes

Immunohistochemistry:
Antiserum was used on perfusion fixed tissue. Perfusion: 1) calcium-free Tyrode's solution, 2) paraformaldehyde-picric acid fixative, and 3) 10% sucrose in PBS as a cryo-protectant. Desired tissues were dissected and stored overnight in 10% sucrose in PBS.

Slide-mounted tissue sections were processed for indirect immunofluorescence. Slides were incubated with blocking buffer for 1 hour at room temperature. Primary antiserum was diluted with blocking buffer to the appropriate working concentration. Blocking buffer was removed and slides were incubated for 18-24 hours at room temperature with primary antiserum. Slides were rinsed 3 times and then incubated with secondary antibodies for 1 hour at room temperature. Slides were again rinsed 3 times and coverslipped. Staining was examined using fluorescence microscopy. (see: PAIN 149 (2010) 393–405).

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Immunocytochemistry:
Antiserum was tested on ASIC3 [DRASIC] transfected cells and primary neuronal cell cultures.

Note: Sodium azide (NaN3) interferes with peroxidase reactions and should not be used with peroxidase methodologies. If sodium azide is present in any steps of the staining procedure, the tissue should thoroughly be rinsed with sodium azide-free buffer before performing the peroxidase reaction.

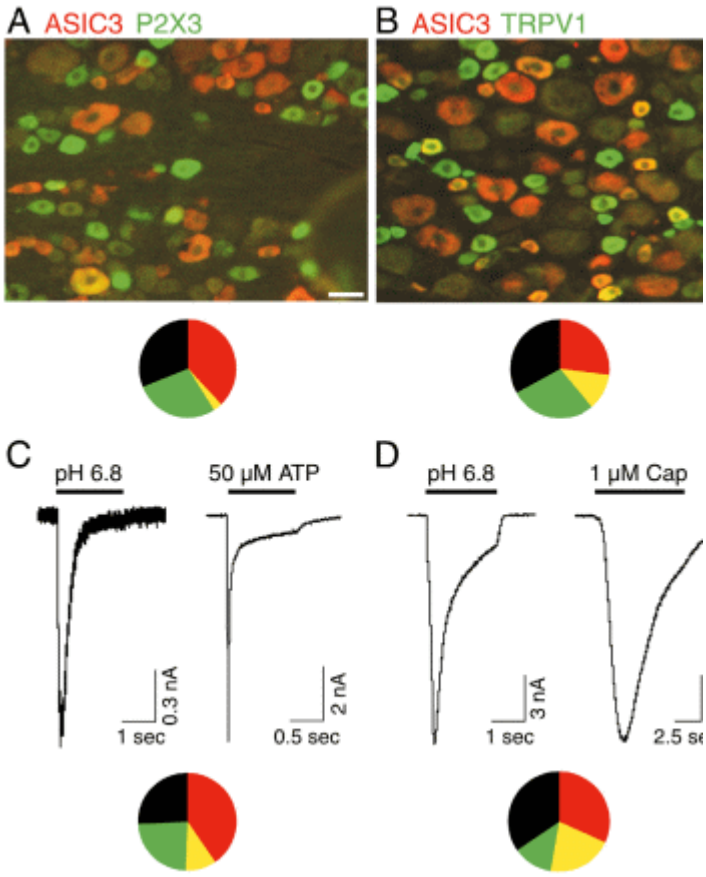
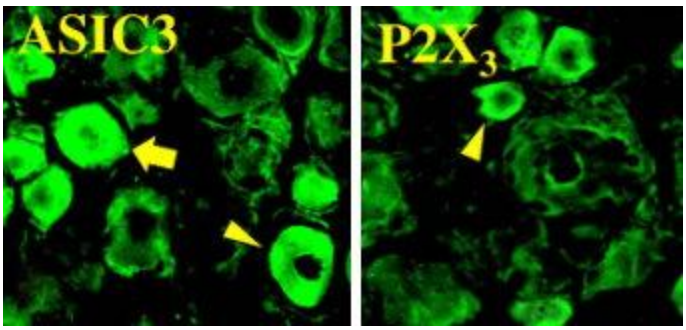


Figure: ASIC3 expression compared to other nociceptive ion channels. DRG sections double-labeled for ASIC3 (red) and either P2X3 (green, A) or TRPV1 (green, B); double-labeled cells are yellow or orange. Circle charts show the relative distribution of the three receptors among all DRG neurons. ASIC3 and P2X3 expression overlapped in only 4% of neurons, while some 35% of ASIC3-positive neurons also express TRPV1. The rare cells positive for both ASIC3 and P2X3 were large (eg. cell at lower left in A). 1406 cells counted for A; 995 for B. C, D, electrophysiological recordings reveal a qualitatively similar expression pattern for currents conforming to ASIC3, P2X3, or TRPV1. The cell that generated the currents in C counts as a co-expressor of ASIC3 and P2X3, fitting into the yellow bin in the circle plot. The cell in D is a co-expressor of ASIC3 and TRPV1. Cells were counted positive if they had at least 0.3 nA of current in response to pH 6.8 (ASIC3+), 50 µM ATP (P2X3+; transient current only), or 1 µM capsaicin (TRPV1). 182 cells recorded for C; 169 for D.



Images: ASIC3 (Dilution 1:10) amd P2X3 (Dilution 1:500) satining of rat Dorsal Root Ganglia (DRGs) of cisplatin-treated animals. After dilution in 0.1 M phosphate-buffered saline (PBS) containing 1.5% normal goat serum and 0.3% Triton X-100 (Sigma), DRG sections were incubated with either guinea pig polyclonal antiserum against synthetic rat ASIC3 and rabbit polyclonal antiserum against synthetic rat P2X3 (1:500; Neuromics). The sections for ASIC3 were reacted with reagents for 2 days at room temperature and others at 4 C. After being rinsed with 0.1 M PBS, the sections were reacted in PBS with fluorescein-isothiocyanate (FITC)-conjugated goat anti-guinea pig or - rabbit IgG antibody (Vector Laboratories, Burlingame, CA, USA) at a concentration of 1:100. After being rinsed with

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0.1 M PBS, the sections were cover-slipped in mounting medium (Immunon, Pittsburgh, PA, USA) and examined under a fluorescence microscope equipped with a digital camera. (see: PAIN 149 (2010) 393–405).

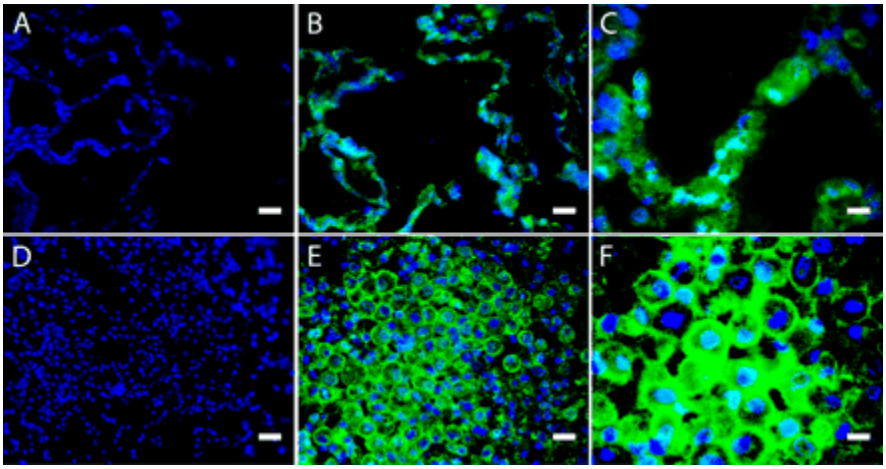

Image: Detection of ASIC3 expression in normal and CF human lung tissues by immunofluorescence microscopy. Normal (top panels A-C) and CF human lung sections (bottom panels D-F) were labeled with a specific antibody against ASIC3 (green channel) and the DNA-selective Hoechst dye (blue channel). A and D, normal and CF human lung sections imaged following incubation with the mixture of anti-ASIC3 antibody and immunopeptide. Shown are normal (B) and CF (E) alveolar structures at a small magnification. ASIC3 expression was detected in both types I and II alveolar cells. Shown are normal (C) and CF (F) alveolar structures at a large magnification. Scale bars equal 10 μm in A, B, D, and E and 20 μm in C and F. These representative images were from 11 sections of four CF lungs. J. Biol. Chem., Vol. 281, Issue 48, 36960-36968, December 1, 2006

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