ASIC3 [DRASIC] 285-304

Data Sheet

Catalog Number: GP14105  
Product Type: Whole Serum  
Host: Guinea Pig  
Species Reactivity: Rat  
Immunogen Sequence: (C)TASLPDDFDPEPSDPLGSP  
Corresponding to residues 285-304 of the extracellular domain rat ASIC3 [DRASIC].

Applications:  
Immunohistochemistry 1:10-1:100  
Immunocytochemistry 1:100

Format: Liquid in phosphate buffered saline (pH 7.4) with 1% bovine serum albumin and 0.05% sodium azide.

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).

Application Notes

References:

FOR RESEARCH USE ONLY

NEUROMICS’ REAGENTS ARE FOR IN VITRO AND CERTAIN NON-HUMAN IN VIVO EXPERIMENTAL USE ONLY AND NOT INTENDED FOR USE IN ANY HUMAN CLINICAL INVESTIGATION, DIAGNOSIS, PROGNOSIS, OR TREATMENT. THE ABOVE ANALYSES ARE MERELY TYPICAL GUIDES. THEY ARE NOT TO BE CONSTRUED AS BEING SPECIFICATIONS. ALL OF THE ABOVE INFORMATION IS, TO THE BEST OF OUR KNOWLEDGE, TRUE AND ACCURATE. HOWEVER, SINCE THE CONDITIONS OF USE ARE BEYOND OUR CONTROL, ALL RECOMMENDATIONS OR SUGGESTIONS ARE MADE WITHOUT GUARANTEE, EXPRESS OR IMPLIED, ON OUR PART. WE DISCLAIM ALL LIABILITY IN CONNECTION WITH THE USE OF THE INFORMATION CONTAINED HEREIN OR OTHERWISE, AND ALL SUCH RISKS ARE ASSUMED BY THE USER. WE FURTHER EXPRESSLY DISCLAIM ALL WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. V3-12/2011

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com
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; 955 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) and P2X3 (Dilution 1:500) staining 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
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