NEUROMICS

P2X3 Data Sheet

Catalog Number:	RA10109	**Host:**	Rabbit
Product Type:	Polyclonal antiserum	**Species Reactivity:**	Human; Rat; Primate
Immunogen Sequence:	VEKQSTDSGAYSIGH Corresponding to residues 383-397 of the carboxy-terminus of rat P2X3	**Format:**	Whole Serum (with 0.05% sodium azide) Sent in liquid form
Applications:	Immunohistochemistry 1:-100-1:300 Immunocytochemistry 1:100-1:300		

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

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

References:

T. Cho, V. V. Chaban. Interaction Between P2X3 and Oestrogen Receptor (ER)α/ERβ in ATPMediated Calcium Signalling In Mice Sensory Neurones. Journal of Neuroendocrinology Volume 24, Issue 5, pages 789–797, May 2012

De Felice, Milena, Sanoja, Raul, Wang, Ruizhong, Vera-Portocarrero, Louis, Oyarzo, Janice, King, Tamara, Ossipov, Michael H., Porreca, Frank. Engagement of descending inhibition from the rostral ventromedial medulla protects against chronic neuropathic pain. PAIN, 152 (12), p.2701-2709, Dec 2011....MOR (rabbit, 1:2000, **Neuromics**, Minneapolis, MN), IB4 (Ab to lectin; 1:1000, Vector Laboratories, Burlingame, CA), P2X3 (rabbit, 1:5000, **Neuromics**)...

B. Huanga, X. Zhaoc, L.-B. Zhengb, L. Zhanga, B. Nia. Different expression of tissue inhibitor of metalloproteinase family members in rat dorsal root ganglia and their changes after peripheral nerve injury. Neuroscience, Volume 193, 13 October 2011, Pages 421-428.

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 (Catalog# P10108) 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 4ºC 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.

Immunocytochemistry: P2X3 transfected cells were processed for indirect immunofluorescence. Media was removed and cells were gently washed 3 times with serum-free media. Following fixation procedure, cells were processed for indirect immunofluorescence as described above.

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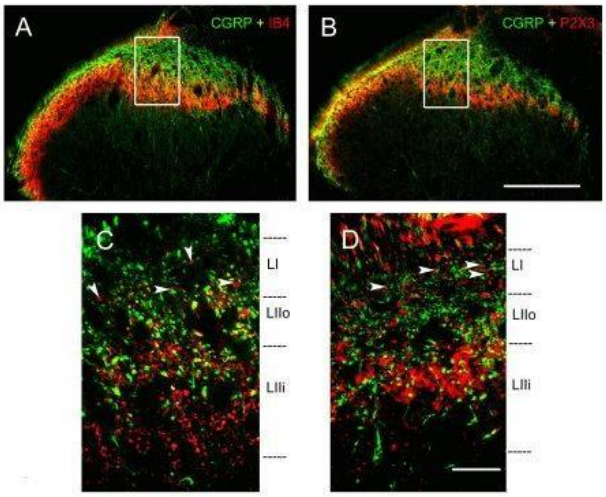
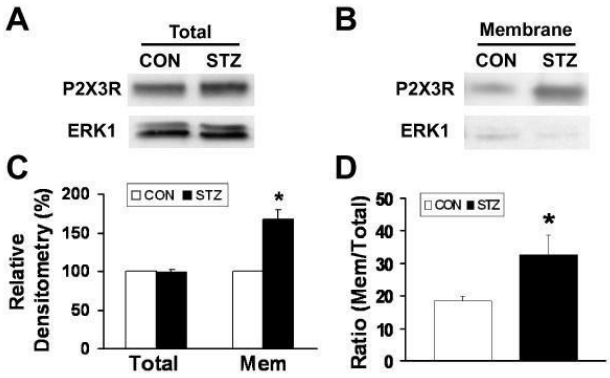
Western Blotting:
Cell membrane extracts were examined by electrophoresis (8% acrylamide) with SDS under reducing conditions and transferred to a nylon membrane. Membranes were blocked for 1 hour at 4°C with 0.1% Tween 20 and 2.5% milk powder (w/v) in PBS. Membranes were incubated with primary antiserum (1:500 in the same buffer overnight at 4°C. Membranes were rinsed and incubated with horseradish peroxidase conjugated secondary antibody for 1 hour at room temperature. Following rinsing, the membranes were processed using enhanced chemiluminescence.

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.



Images: CGRP, IB4 and P2X3 staining in transverse spinal cord sections. A and B show low magnification confocal images of CGRP-IR and IB4 positive (A) or P2X3-IR (B) fibers. C and D represent high magnification confocal images from the middle third of the lateromedial extent of the superficial dorsal horn. In C, note that there is limited co-localization of IB4 and CGRP (in yellow). Arrowheads show axonal varicosities (boutons) from nonpeptidergic fibers in lamina I, which do not co-localize CGRP immunoreactivity. The framed regions in A and B indicate the approximate regions from where C and D, respectively, were obtained (the latter originate from other sections). CGRP (in green); IB4 (in red); P2X3 (in red). Scale bar (A, B) = 200 µm; scale bar (C, D) = 20 µm. Molecular Pain 2012, 8:64 doi:10.1186/17448069-8-64.



*Images: **STZ treatment enhances the membrane protein expression of P2X3Rs**. (A) Total and (B) membrane expression of P2X3R proteins in DRGs of control and STZ-treated rats. The total P2X3R protein expressions were normalized with their respective ERK1. The low expression of ERK1 in membrane fractions suggests a minimal contamination of cytoplasmic P2X3Rs in protein samples. (C) The total expression of P2X3Rs did not change with STZ treatment. On the other hand, the expression of membrane P2X3Rs was significantly increased (*P < 0.05, n = 5). (D) Membrane/total ratio of P2X3R expression. The membrane expression of P2X3Rs was normalized to the total P2X3R expression in its respective rat group. The membrane/total P2X3R expression ratio in control rats was 18.1 ± 0.8% (n = 5) in control rats and was 33.6 ± 5.8% (n = 5) in STZ rats. The membrane expression of P2X3Rs was significantly enhanced with STZ treatment (P < 0.05). Xu et al. Molecular Pain 2011 7:60 doi:10.1186/17448069-7-6.*

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