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Product datasheet for GP14106-50

P2X2 (P2RX2) Guinea Pig Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	IF, IHC, WB
Recommend Dilution:	Immunohistochemistry: 1:500. Immunocytochemistry: 1:500. Western Blot: 1:500.
Reactivity:	Human, Monkey, Rat
Host:	Guinea Pig
Clonality:	Polyclonal
Immunogen:	Corresponding to residues 460-472 of the carboxy-terminus of rat P2X2.
Specificity:	This antibody reacts to P2X2.
Formulation:	State: Serum State: Liquid serum containing 0.05% sodium azide
Gene Name:	purinergic receptor P2X 2
Database Link:	Entrez Gene 22953 Human
Background:	P2X2 is a subunit in the family of ion channels activated by extracellular ATP. It was cloned from PC12 cells. P2X2 has two putative transmembrane domains with intracellular N- and C-termini. It can form homomeric channels and heteromeric channels with other members of the family. P2X2 is expressed in brain, spinal cord, sensory and autonomic ganglia as well as in neuroendocrine cells.
Synonyms:	P2X purinoceptor 2, ATP receptor, Purinergic receptor

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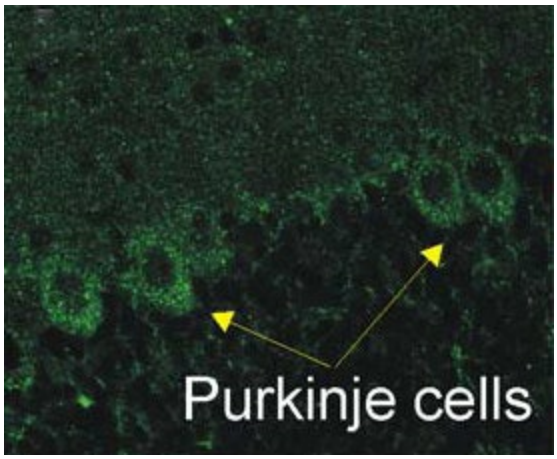
Note: Sodium azide (NaN3) interferes with peroxidase reactions and should not be used with peroxidase methodologies. If sodium azide is present in any steps of the staining procedure, the tissue should thoroughly be rinsed with sodium azide-free buffer before performing the peroxidase reaction.

Protocol: Immunohistochemistry:
Antiserum was used on perfusion fixed tissue. Perfusion:
1) calcium-free Tyrodes 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 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.

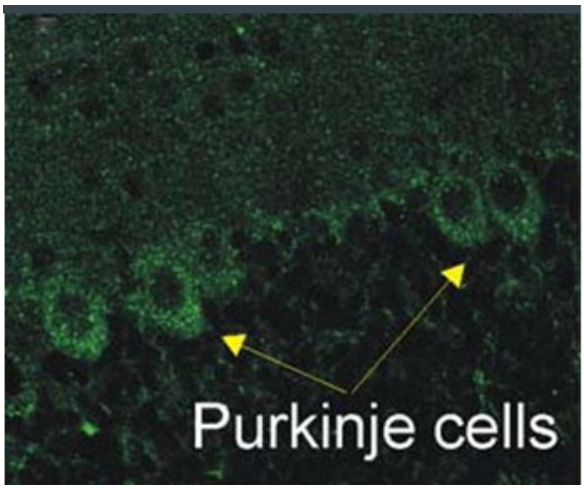
Immunocytochemistry:
P2X2 transfected cells were processed for indirect immunofluorescence. Media was removed and cells were gently washed 3 times with serum-free media. Following fixation procedure, cells were processed for indirect immunofluorescence as described above.

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.

Product images:



P2x2 staining of Cerebellar Purjinke cell layer. ~50 µm sections from 3 brains from P15 rats. http://stke.sciencemag.org/cgi/data/sigtrans;1/41/ra8/DC1/1



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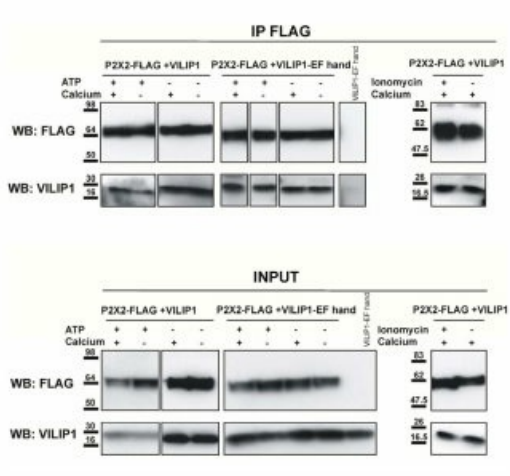
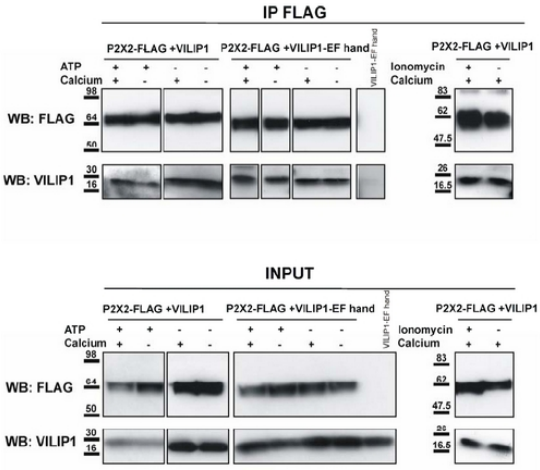


The constitutive interaction between P2X2 receptors and VILIP1 is calcium independent. Calcium dependence of the interaction between P2X2 receptors and VILIP1 was assessed by coimmunoprecipitation from HEK cells expressing P2X2-FLAG receptors and either VILIP1or VILIP1 EF-hand mutants. The effect of calcium on the interaction was tested before cell lysis by increasing intracellular calcium with ATP application or ionomycin treatment, and also during the immunoprecipitation steps. In the latter case, experiments were carried in the absence of calcium and in the presence of 5 mM EDTA or in the absence of EDTA and in the presence of 500µM calcium. P2X2 receptors were immunoprecipitated using a FLAG antibody conjugated to agarose beads. Bound proteins were eluted by competition using a FLAG peptide. As shown in the top panel, no difference in P2X2-FLAG-VILIP1 interaction was observed in the presence or the absence of calcium in the immunoprecipitation buffer. In addition, stimulation of transfected cells with ATP (100µM, 5 minutes) or with ionomycin (2µM, 5 minutes) prior to lysis did not affect the interaction between the two proteins. A similar observation was made when the VILIP1 EF hand mutant was co-immunoprecipitated. No immunoprecipitation was observed when VILIP1 was transfected alone. The bottom panel shows the total protein input used for immunoprecipitation.
www.sciencesignaling.org/cgi/content/full/1/41/ra8/DC1



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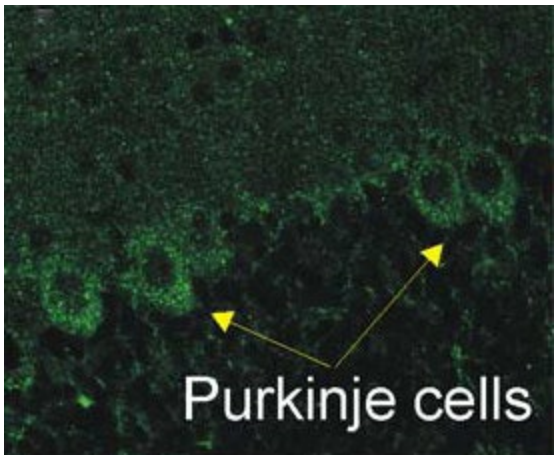

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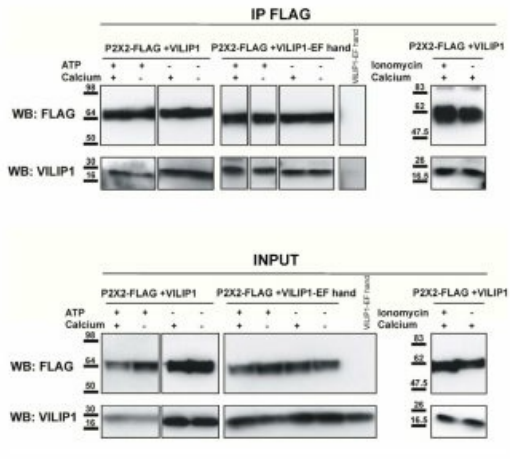

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