NEUROMICS

P2X2 Data Sheet

Catalog Number:	GP14106	**Host:**	Guinea Pig
Product Type:	Polyclonal antiserum	**Species Reactivity:**	Rat, human
Immunogen Sequence:	DSTSTDPKGLAQL Corresponding to residues 460-472 of the carboxy-terminus of rat P2X2	**Format:**	Whole Serum (with 0.05% sodium azide) Sent in liquid form

Applications: Immunohistochemistry 1:500
Immunocytochemistry 1:500
Western Blotting 1:500
Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted serum and immediately place at -20ºC. Serum may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20ºC. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

References:

Brake, A. J., Wagenbach, M. J., and Julius, D. (1994). *New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor.* Nature *371,* 519-23.

Vulchanova, L., Arvidsson, U., Riedl, M., Wang, J., Buell, G., Surprenant, A., North, R. A., and Elde, R. (1996). *Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry.* Proc Natl Acad Sci U S A *93,* 8063-7.

Vulchanova, L., Riedl, M. S., Shuster, S. J., Buell, G., Surprenant, A., North, R. A., and Elde, R. (1997). *Immunohistochemical study of the P2X2 and P2X3 receptor subunits in rat and monkey sensory neurons and their central terminals.* Neuropharmacology *36*, 1229-42.

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 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.

*Not*e: Sodium azide (NaN_3) 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.

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