NEUROMICS

PGP9.5 Datasheet

Catalog Number:	RA12103	Host:	Rabbit
Product Type:	Polyclonal antiserum	Species Reactivity:	Human, Rat, Mouse, Pig
Immunogen Sequence:	ASSEDTLLKDAAKVCR	Format:	Whole Serum (with 0.05% sodium azide) Sent in liquid form

Applications: Immunohistochemistry 1:100-1:500
Western Blotting 1:1000
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:

Day, I. N., Hinks, L. J., and Thompson, R. J. (1990). *The structure of the human gene encoding protein gene product 9.5 (PGP9.5), a neuron-specific ubiquitin C-terminal hydrolase.* Biochem J *268*, 521-4.

Navarro, X., Verdu, E., Wendelschafer-Crabb, G., and Kennedy, W. R. (1997). *Immunohistochemical study of skin reinnervation by regenerative axons.* J Comp Neurol *380,* 164-74.

Application Notes

Immunohistochemistry:
Antiserum was used on perfusion fixed tissue. Perfusion: 1) calcium-free Tyrode's solution, 2) 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.

Western Blotting:
Solubilized human brain extract was examined by SDS-PAGE (4-20% tris-glycine gel) under reducing conditions. The gel was transferred to a nitrocellulose membrane and blocked overnight at room temperature with casein blocking buffer. Following blocking, primary antiserum (1:2000 in 1%BSA, PBS) was added for 4 hours at room temperature. Membranes were then washed and incubated with alkaline phosphatase conjugated secondary antibody for 45 minutes at room temperature. After washing the membranes were treated with BCIP/NBT substrate reagents to visualize immobilized protein.

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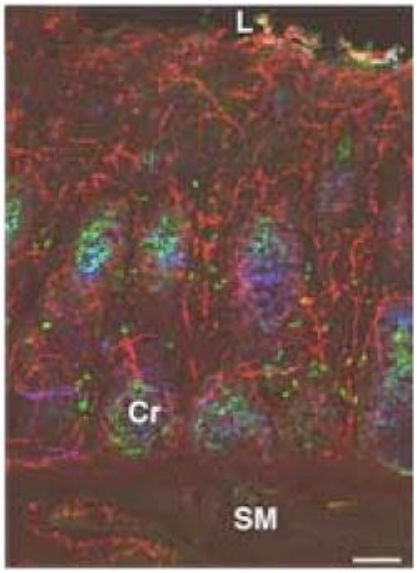
Note: Also tested positive for western blot using mouse hippocampal lysates where a primary band is seen between 25 and 30 kDa.

Description/Data:

PGP9.5 is a soluble cytoplasmic protein with a molecular weight of approximately 25,000 kD. It is present in neurons and in cells of the diffuse neuroendocrine system. PGP9.5 functions as a tissue-specific ubiquitin carboxyl terminal hydrolase isoenzyme. This enzyme is also known as UCH-L1. Because of its abundance in nerves, it has been widely used as a marker for peripheral nerve fibers.

It was also discovered as a gene mutated in some rare famial forms of Parkinson's disease. Interestingly a common allelic variant of UCHL1, the S18Y polymorphism is actually protective against Parkinson's disease. It is also a marker for cells of the diffuse neuroendocrine system and their tumors.



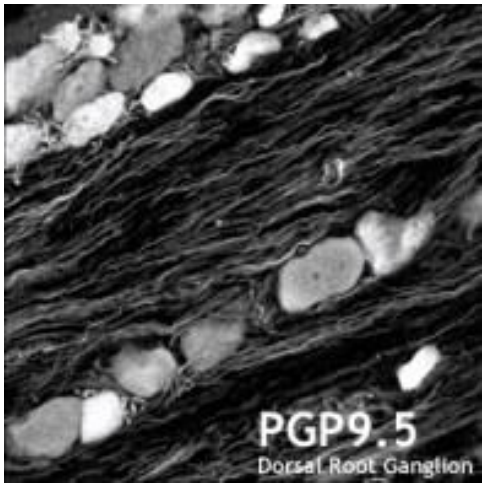


Image: PGP9.5 staining of epithelial cells in porcine distal colon. Nerve fibers immunoreactive for the neural marker PGP 9.5 (red) are in close proximity to both IgAimmunoreactive plasma cells (green) and epithelial cells immunoreactive for SC (blue) in the colonic crypts. Abbreviations: Cr, colonic crypt; L, intestinal lumen; SM, submucosa. Scale bars: A, 100 µm. J Neuroimmunol. Published online 2007 February 21. doi: 10.1016

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