Datasheet for ABIN1742304

anti-SLC18A3 antibody (C-Term)

1 Validation | 3 Images | 4 Publications

Go to Product page

Overview

Quantity:	50 µg
Target:	SLC18A3
Binding Specificity:	AA 475-530, C-Term
Reactivity:	Mouse, Rat
Host:	Guinea Pig
Clonality:	Polyclonal
Conjugate:	This SLC18A3 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC)

Product Details

Immunogen:	Strep-Tag® fusion protein of the C-terminal part of rat VAChT (aa 475-530).
Specificity:	Specific for VAChT.
Purification:	Affinity purified with the immunogen. Guinea pig serum albumin was added for stabilization.

Target Details

Target:	SLC18A3
Alternative Name:	VAChT (SLC18A3 Products)

Application Details

Application Notes:	WB: 1 : 500 up to 1 : 1000 (AP staining)

Application Details

	IP: not tested yet ICC: not tested yet IHC: 1 : 100 up to 1 : 300
Comment:	This antibody is less sensitive compared to the rabbit antibody. VAChT aggregates after boiling, making it necessary to run SDS-PAGE only with non-boiled samples.
Restrictions:	For Research Use only

Handling

Format:	Lyophilized
Reconstitution:	For reconstitution add 50 µL H2O to get a 1mg/ml solution of antibody in PBS. Then aliquot and store at -20 °C until use.
Buffer:	PBS
Handling Advice:	Affinity purified antibodies are less robust than antisera, since protease inhibitors are also removed during purification. Hence, storage at 4 °C for prolonged periods (i.e. several weeks), is not recommended.
Storage:	-20 °C
Storage Comment:	Unlabeled lyophilized antibodies are stable in this form without loss of quality at ambient temperatures for several weeks or even months. They can be stored at 4°C for several years. Lyophilized antibodies must not be stored in the freezer, they may be destroyed!

Publications

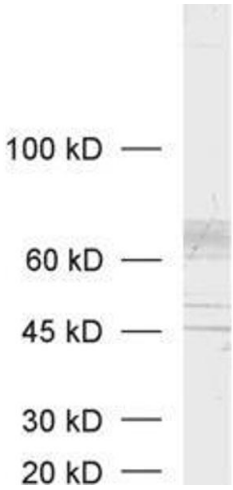
Product cited in:	Cao, Gou, Du, Fan, Liang, Yan, Lin, Jin, Du: "Glutamatergic and central cholinergic dysfunction in the CA1, CA2 and CA3 fields on spatial learning and memory in chronic cerebral ischemia-Induced vascular dementia of rats." in: **Neuroscience letters**, Vol. 620, pp. 169-176, (2016) (PubMed). Downs, Jalloh, Prater, Fregoso, Bond, Hampton, Hoover: "Deletion of neurturin impairs development of cholinergic nerves and heart rate control in postnatal mouse hearts." in: **Physiological reports**, Vol. 4, Issue 9, (2016) (PubMed). Rodella, Scorzeto, Duregotti, Negro, Dickinson, Chang, Yuki, Rigoni, Montecucco: "An animal model of Miller Fisher syndrome: Mitochondrial hydrogen peroxide is produced by the

Publications

autoimmune attack of nerve terminals and activates Schwann cells." in: **Neurobiology of disease**, Vol. 96, pp. 95-104, (2016) (PubMed).

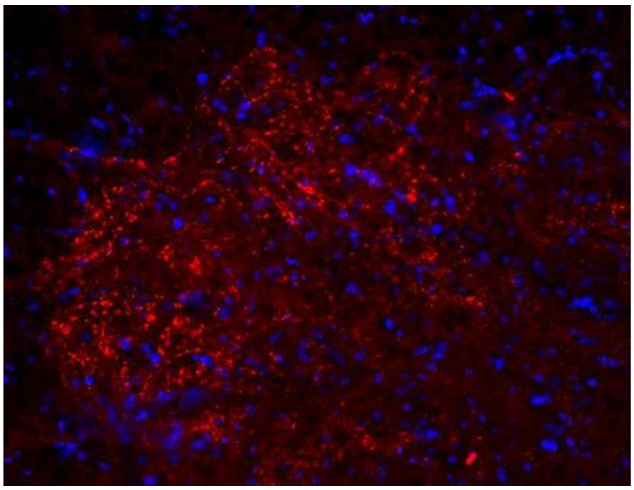
Gallart-Palau, Tarabal, Casanovas, Sábado, Correa, Hereu, Piedrafita, Calderó, Esquerda: " Neuregulin-1 is concentrated in the postsynaptic subsurface cistern of C-bouton inputs to α-motoneurons and altered during motoneuron diseases." in: **FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, Vol. 28, Issue 8, pp. 3618-32, (2014) (PubMed).

Images



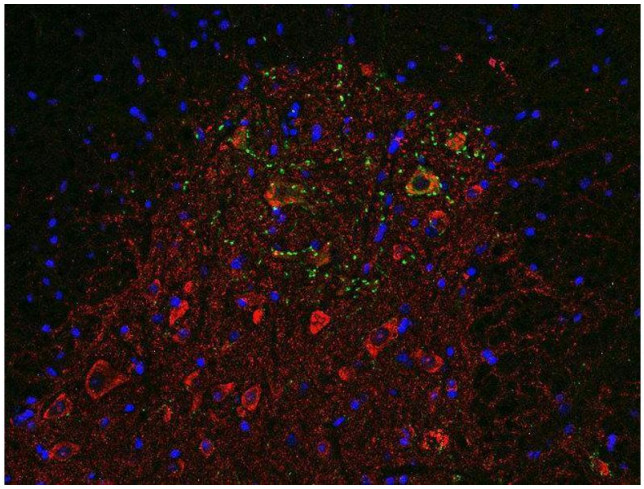
Western Blotting

Image 1. dilution: 1 : 1000, sample: synaptic vesicle fraction of rat brain (LP2)



Immunohistochemistry

Image 2. Indirect immunolabeling of mouse spinal cord (dilution 1 : 100; red). Nuclei have been visualized by DAPI staining (blue).



Immunohistochemistry (Paraffin-embedded Sections)

Image 3. Indirect immunofluorescence labeling of PFA fixed, paraffin embedded mouse spinal cord section with anti-VAchT (dilution 1 : 500; green)and rabbit anti-SV 31 (cat. no. 228 002, dilution 1 : 500; red). Nuclei have been visualized by DAPI staining (blue).

Successfully validated (Immunofluorescence (IF))

by Martinelli Lab, Neuroscience Department, UConn Health

Report Number: 101568

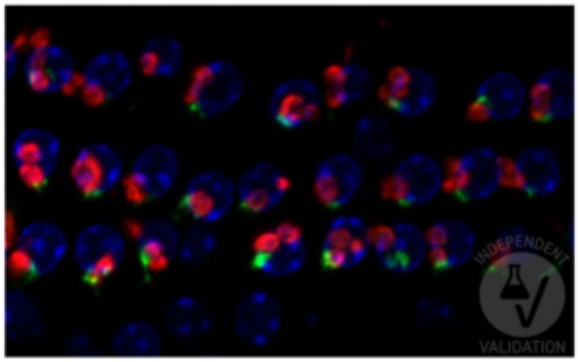
Date: Sep 05 2017

Target:	SLC18A3
Lot Number:	139105/6
Method validated:	Immunofluorescence (IF)
Positive Control:	Organ of Corti tissue dissected from the cochlea of the inner ear, from a WT mouse, C57BL/6 strain, 10 weeks old
Negative Control:	Primary and secondary only antibody controls
Notes:	Passed. ABIN1742304 specifically recognizes SLC18A3 with little background signal in murine organ of Corti tissue dissected from the cochlea of the inner ear.
Primary Antibody:	ABIN1742304
Secondary Antibody:	goat anti-guinea pig AF633 conjugated antibody (Life Technologies, A21105, lot 1812311)

Protocol:	• Dissect organ of Corti from the mouse cochlea in the inner ear as described in Maison, Liberman, and Liberman (2016).
	• Cryoprotect and freeze/thaw to permeabilized the tissue:
	  ◦ Transfer cochlear pieces to a 5ml disposable cup with approximately 1ml of 30% sucrose in 100mM phosphate buffer (PB) at RT.
	  ◦ Incubate tissue on a shaker for 15min at RT.
	  ◦ Wash tisse with 30% sucrose in PB at RT.
	  ◦ Incubate tissue on a shaker for 15min at RT.
	  ◦ Place cup on dry ice untill contents freeze completely.
	  ◦ Allow cup to thaw at RT.
	  ◦ Pipet out the sucrose solution and wash tissue 3x for 15min with PBS containing 0.1% triton X-100 on a shaker at RT.
	• Block tissue with 5% goat serum containing 0.3% Triton-X on a shaker for 30-60 min at RT.
	• Pipet out PBS + detergent and add blocking solution (supplier, product no, lot no).
	• Cut cap off a 1.5ml microcentrifuge tube and transfer pieces in the blocking solution to the flipped upside-down cap.
	• Incubate tissue in the flipped upside-down caps with 100µl primary
	  ◦ guinea pig anti-SLC18A3 antibody (antibodies-online, ABIN1742304, lot 139105/6) diluted 1:300

	○ chicken anti-Parvalbumin (Synaptic Systems, 195006) diluted 1:400 • in blocking solution ON at RT on an agitator. Fasten tubes onto the caps and and protect them from the light. • Pipet out the primaries. • Rinse tissue 3x for a total of 10 min with PBS containing 0.1 % Triton X-100. • Incubate tissue in the flipped upside-down caps with 100µl secondary ○ goat anti-guinea pig AF633 conjugated antibody (Life Technologies, A21105, lot 1812311) ○ goat anti-chicken AF488 conjugated antibody (Life Technologies, A11039, lot 1812246) • diluted 1:300 in blocking solution for 1h at RT away from the light. • Rinse tissue 3x for a total of 10min with PBS. • Transfer tissue pieces onto a slide with stereocilia facing up. • Add mounting Fluoromount-G with DAPI mounting medium (ThermoFisher Scientific, 00-4959-52, lot B2215-N915) then coverslip. • Image acquisition on a Zeiss Axiovert epifluorescence with with a 63x objective, using a Zeiss ApoTome for optical sectioning.
Experimental Notes:	• The observed signal on outer hair cells in the organ of Corti for ABIN1742304 appears indistinguishable to the published and expected signal documented in numerous publications (see e.g. figure 4 in Maison, Liberman, and Liberman (2016)). • No signal was observed in either of the negative controls. ABIN1742304 worked very well at 1:300 dilution. It could probably be further diluted and still achieve acceptable signal to noise ratio. • No signal was observed with the secondary antibody only negative control.

Image for Validation report #101568



Validation image no. 1 for anti-Solute Carrier Family 18 (Vesicular Acetylcholine), Member 3 (SLC18A3) (AA 475-530), (C-Term) antibody (ABIN1742304)

In this photograph of the 3 rows of cochlear outer hair cells taken with 63x objective, nuclei are labeled with DAPI (blue), SLC18A3 is labeled with ABIN1742304 (red), and green marks the parvalbumin signal. Note that SLC18A3-positive synapses are adjacent to the parvalbumin-positive synapses, as expected since SLC18A3 marks efferent synapses and parvalbumin marks afferent synapses. These two synapses are known to be adjacent on outer hair cells.