

HUMAN SODIUM ION CHANNEL Na_v1.7

2017

Protein information

Target Name	Human sodium ion channel Na _v 1.7
Catalogue Number	PP5
Class	Sodium Ion Channel
Sequence	<p>Full-length, wildtype sequence, with a C-terminus TEV cleavage site, 10xHis-tag and 2xStrep-tag II:</p> <p>MAMLPPPGPQSFVHFTKQSLALIEQRIAERKSKEPKKEKKDDDEEAPKPSSDLEAGKQLPFIYGDIPP GMVSEPLEDLDPPYADKKTIVLNKGGKTIFRFNATPALYMLSPFSPRRISIKILVHSLFSMLIMCTILT NCIFMTMNNPPDWTKNVEYFTFTGIYTFESLVKILARGFCVGEFTFLRDPWNWLDFFVIVFAYLTFEVDN LGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKLLSDVMILTVFCLSVFALIGLQLFMGNLKHKCF RNSLENNETLESIMNTLESEEDFRKYFYYLEGSKDALLCGFSTDSGQCPEGYTCVKIGRNPDIYGYTSF DTFSWAFALFRLMTQDYWENLYQQTLRAAGKTYMIFVVFVIFLGSFYLINLILAVVAMAYEEQNQAN IEEAKQKELEFQQMLDRLKKEQEEAEIAAAAAEYTSIRRSRIMGLSESSSETSLSKSSAKERRNRR KKKNQKLLSSGEEKGDAEKLSKSESEDSIRRSKSFHLGVEGHRAHEKRLSTPNQSPLSIRGSLFSAR RSSRTSLFSFKGRGRDIGSETEFADDEHSIFGDNESRRGSLFVPHRPQERRSSNISQASRSPMLPV NGKMMSAVDCNGVSLVDGRSALMLPNGQLLPEGTTNQHKKRRCSSYLLEDMLNDPNLRQRAM SRASILTNTVEELEESRQKCPPWWYRFAHKFLIWNCSPIYKFKKCIYFIVMDPFVDLAITICIVLNTL FMAMEHHPMTEEFKNVLAIGNLVFTGIFAAEMVLKLIAMDPYEFYQVGNWIFDLSLIVTSLVFLADV EGLSVLRFRLLRVFKLAKSWPTLNMLIKIIGNSVAGLGNLTLVLAIIIVFIFAVVGMQLFGKSYKECVC KINDDCTLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCMEVAGQAMCLIVMMVMVIGNLVVNLNLF LALLSSFSNDLTAIEEDPDANNLQIAVTRIKKGINYVKQTLREFILKAFSKPKISREIRQAEDLNTK KENYISNHTLAEMSKGHNFLKEKDKISFGSSVDKHLMEDSDGQSFHNPSTLTVTVPIAPGESDLEN MNAEELSSDSDSEYSKVRNLRSSSSECSTVDNPLPGEGEEAEAEPMNSDEPEACFTDGCVRRFSCC QVNIESGKGIWWNIRKTCYKIVEHSWFESFIVLMILLSSGALAFEDIYERKTKIKIILEYADKIFTYIF ILEMLLKWIAYGKTYFTNAWCWLDLIVDVSLVTLVANTLGYSDLGPIKSLRTLRLRPLRALSFRFEG MRVVVNALIGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGKFYECINTTDGSRFPASQVPRSECFALMN VSQNVRWKLNKLVNFDNVGLGYSLLQVATFKGWTIIMYAAVDSVNVDKQPKYEYSLYMYIYFVVFII FGSFFTLNLFIVGIIIDNFNQKKKLGQDIFMTEEQKKYYNAMKKLGSKPKQPIPRPGNKIQGCIFD LVTNQAFDISIMVLIICLNMTMMVEKEGQSQHMTEVLYWINVVFIIIFTGECVLKLIISLRHYFTVGV NIFDFVVIISIVGMFLADLIETYFVSPTLFRVIRLARIGRILRLVKGAKGIRTLFALMMSLPALFNIGLL LFLVMFIYAIFGMSNFAYVKKEDGINDMFNFETFGNSMICLFQITTSAGWDGLLAPILNSKPPDCDPK KVHPGSSVEGDCGNPSVGIFVFSYIIISFLVVVNMVIAVILENFSVATEESTEPLSEDDFEMFYEVWE KFDPDATQFIEFSKLSDFAAALDPLLIAKPNKVQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEM DSLRSQMEERFMSANPSKVSYPITTTLLKRKQEDVSATVIQRAYRRYRLRQNVKNISSIYIKDGDRD DILLNKKDMAFDNVNENSSPEKTDATSSSTTSPSYDSVTKPDKEKYEQDRTEKEDKGGKDSKESKKE NLYFQGHHHHHHHHHHGAWSHPQFEKGGGSGGGSSGSAWSHPQFEK</p>
Affinity Tag	10xHis, 2xStrep-tag II
Origin	Human (Homo sapiens)
Theor. MW	230kDa
Accession #	NP_002968.1 (UniProt)



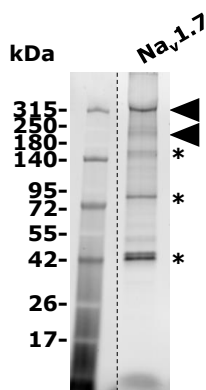
Protein production

Expression system	BacMAM expression system in CHO-S cells
Purification	Immobilized Metal Affinity Chromatography and Size Exclusion Chromatography
Purity	~25%
Activity	ProTx-II saturation binding assay (see below)
Concentration	Up to 5mg/mL
Sample Buffer	25mM Na ₂ HPO ₄ , 150mM NaCl, 0.4% sarkosyl, 0.004% CALX07gk, 10% glycerol
Available quantity	From 10µg up to mg scale
References	1- Vetter I. et al. NaV1.7 as a pain target - From gene to pharmacology. <i>Pharmacol. Ther.</i> 2017; 172, 73–100. 2- Desuzinges Mandon E. et al. Novel systematic detergent screening method for membrane proteins solubilization. <i>Anal Biochem.</i> 2017 Jan 15; 517:40-49.

Miscellaneous

Shipment Temperature	Dry ice
Storage conditions	Store at -80°C

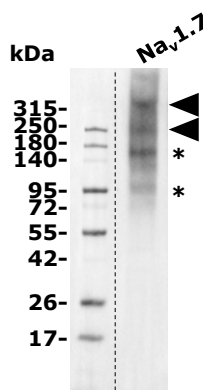
Quality Controls



SDS-PAGE, 4-15% acrylamide gel
Bio-Rad Stain-Free™ detection

SDS-PAGE, Stain-Free™ detection

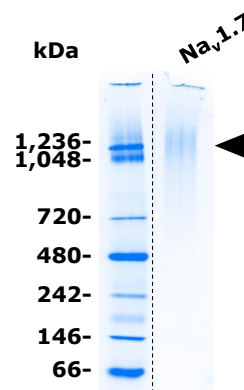
Purified Na_v1.7 was migrated on a 4-15% Tris-glycine SDS-PAGE and the total proteins were Stain-Free detected. Black arrows indicate the target. * Potential degradation of structural domains of Na_v1.7.



SDS-PAGE, 4-15% acrylamide gel
WB anti-Na_v1.7 N68/6 (1:200)

SDS-PAGE, western blotting

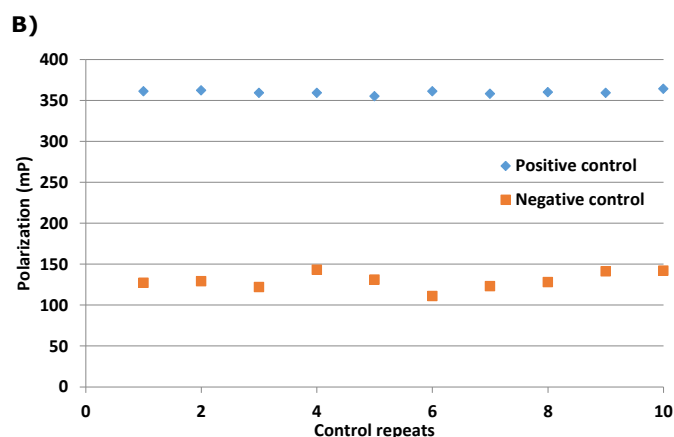
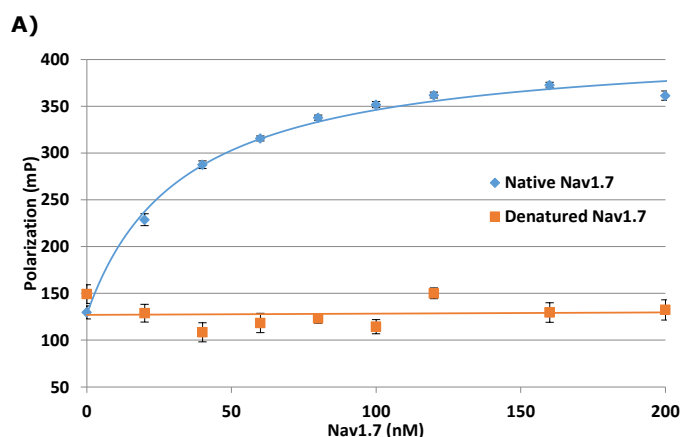
Purified Na_v1.7 was migrated on a 4-15% Tris-glycine SDS-PAGE, transferred to pvdf membrane and immunodetected with an anti-Na_v1.7 N68/6 antibody (Merck Millipore, cat#MABN41). Black arrows indicate the target. * Potential degradation of structural domains of Na_v1.7.



Native-PAGE, 3-12% acrylamide gel
Coomassie blue staining

Native-PAGE, Coomassie blue staining

Purified Na_v1.7 was migrated on a 3-12% Bis-Tris Native-PAGE and the total proteins were stained with Coomassie blue. Black arrow indicates the target.



ProTx-II saturation binding assay

A) The polarization values of 400nM Cy5-ProTx-II were measured after incubation for 90min at RT in presence of a range of concentrations from 0 to 200nM of native Na_v1.7 (in blue) or denatured Na_v1.7 (in orange). Na_v1.7 was denatured by heating the purified protein at 98°C for 15min. Lower concentrations of protein and ligand could not be explored due to the limited sensitivity of the measurement, preventing the determination of kinetic constants such as the K_d. Positive controls in blue (10 replicates) were 400nM Cy5-ProxTx-II with 200nM Na_v1.7 in PBS buffer. Negative controls in orange (10 replicates) were 400nM Cy5-ProxTx-II in PBS buffer. The Z'-factor was determined using the polarization values from 10 repeats of negative and positive controls. The Z'-factor for the measurement was 0.85 and is an indication of a good performance for the assay.

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