

B'SYS GmbH

HEK-293 Nav1.8

Specification Sheet

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TABLE OF CONTENTS

1. BACKGROUND	3
1.1 Voltage-gated Sodium channel Nav1.8	3
2. VALIDATION OF HEK-293 NAV1.8 CELLS	3
2.1 Biophysical characterization	3
2.2 Pharmacological characterization	4
2.3 Automated Patch-clamp	5
3. NAV1.8 SEQUENCE	5
3.1 Human Nav1.8 (SCN10A)	5
3.2 Human Nav1.8 Beta 3 (SCN3B)	5
4. CONTACT INFORMATION	6
4.1 Contact Address for Technical Support & Ordering Information	6

1. BACKGROUND

1.1 Voltage-gated Sodium channel Na_v1.8

Human Na_v1.8 (SCN10A) is a TTX resistant voltage-gated Na⁺ channel only expressed in the peripheral sensory nervous system. It is involved in transmitting nociceptive information to the central nervous system and is therefore an interesting target to treat neuropathic pain.

2. VALIDATION OF HEK-293 NA_v1.8 CELLS

2.1 Biophysical characterization

Na_v1.8 currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137 mM KCl 4 mM, CaCl₂ 1.8 mM, MgCl₂ 1 mM, HEPES 10 mM, D-Glucose 10 mM, pH (NaOH) 7.4. The pipette solution consisted of (in mM) KCl 120 mM, NaCl 10 mM, MgCl₂ 6 mM, HEPES 10 mM, EGTA 5 mM, pH (KOH) 7.2. After formation of a GΩ seal between the patch electrodes and individual Na_v1.8 stably transfected HEK-293 cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. As soon as a stable seal could be established fast deactivating sodium currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV up to +60 mV in 10 mV increments of 100 ms duration (Fig. 1 A, B). The voltage pulses were run at intervals of 10 s.

For the IV curve the peak current amplitude of the pulses between -80 mV and +60 mV was plotted versus the applied voltage. This resulted in a bell shaped curve. The minimum of the IV curve was between +10 mV and +20 mV (Fig 1 B).

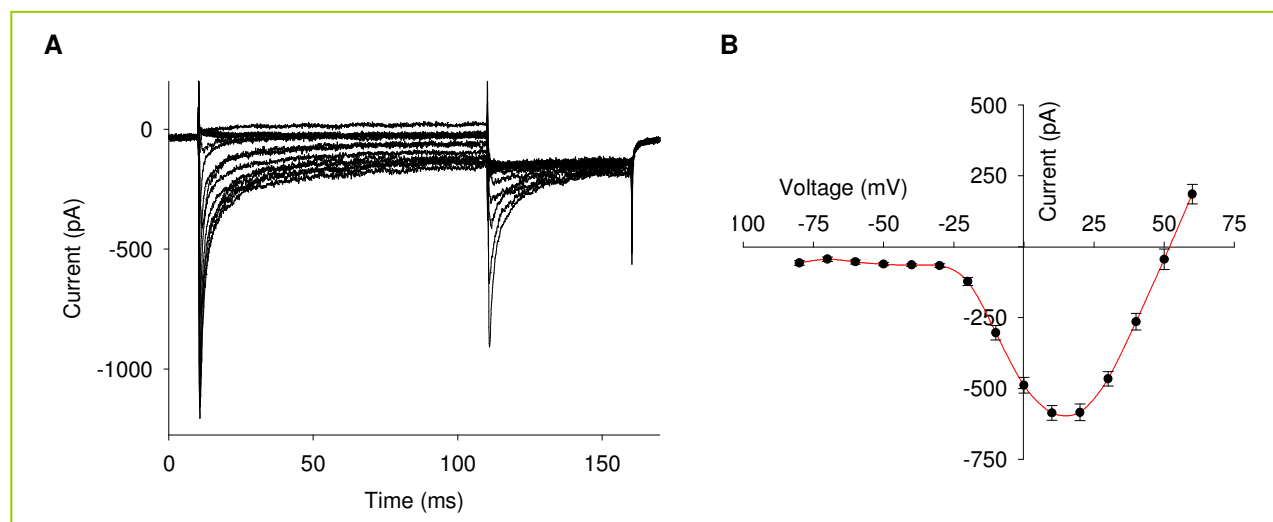


Fig. 1: IV curve of stably transfected HEK-293 Na_v1.8 cells. A) Representative current recording. Depolarized to potentials between -80 mV and +60 mV in 10 mV increments from a holding potential of -80 mV. B) IV curve of the Na_v1.8 channel. Peak currents were measured at the beginning of the voltage pulse (n=8). No currents were recorded in untransfected cells.

Tab. 1: Biophysical Properties of HEK-293 Nav1.8 cells

Channel	Expression System	Averaged Current	Proposed potentials (mV)		
			Resting state	Half inactivated	Max peak
Nav1.8 (SCN10A)	HEK-293	586 ± 25 pA n=8	-130mV	$V_{0.5} = -52.53 \pm 3.05$ mV $k = -10.45 \pm 1.09$ (n=8)	$I_{max} = 15$ mV (n=8)

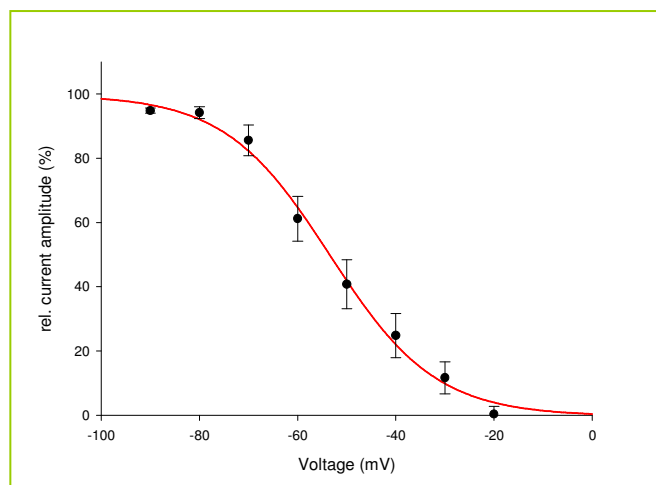
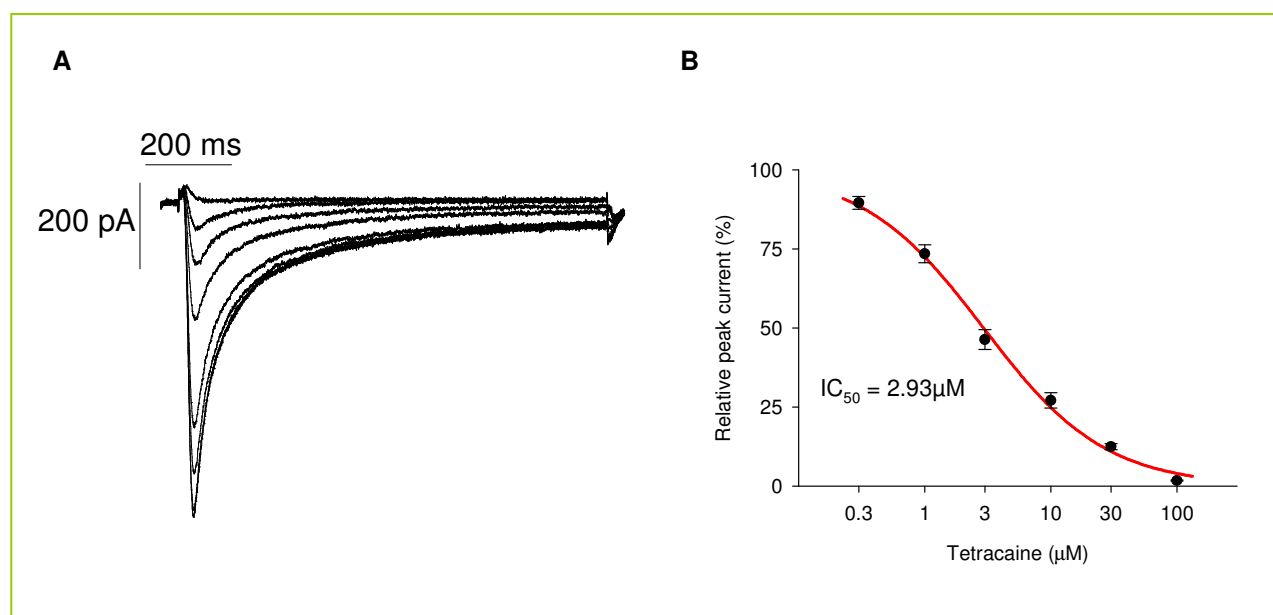


Fig. 2: Inactivation curve of Nav1.8

2.2 Pharmacological characterization

For the pharmacological characterization of the Nav1.8 channel Tetracaine was tested at concentrations of 0.3, 1.0, 3.0, 10, 30 and 100 μM ($n \geq 3$ cells). The dose response curves was generated (see Fig. 3) and the IC_{50} values calculated.

The Nav1.8 currents were stimulated by a 1000 ms pulse to 10 mV from a holding potential of -80 mV.

**Fig. 3:** Dose response curves for Tetracaine @ -80 mV: A: representative current recording, B: Dose Response curve

2.3 Automated Patch-clamp

The HEK-293 Na_v1.8 cell line was validated on the automated patch-clamp system Q-Patch 16. The bath solution contained NaCl 137 mM, KCl 4 mM, CaCl₂ 1.8 mM, MgCl₂ 1 mM, HEPES 10 mM, D-Glucose 10 mM, pH (NaOH) 7.4. The intracellular solution consisted of CsF 135 mM, NaCl 10 mM, HEPES 10 mM, EGTA 5 mM, pH (CsOH) 7.3. Cells were clamped at -110 mV and depolarized to 0 mV for 200 ms every 10 s. Increasing concentrations of Tetracaine were applied and the resulting IC₅₀ value was determined to be 24.42 μM.

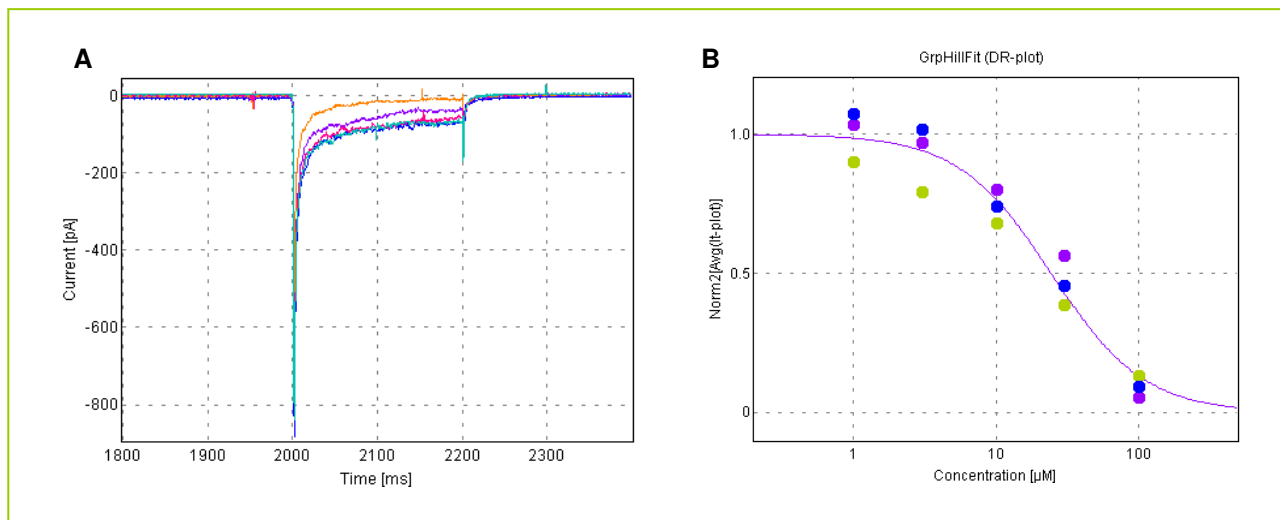


Fig. 4: Dose response curves for Tetracaine @ -110 mV. A: representative current recording, B: dose response curve.

3. NA_v1.8 SEQUENCE

3.1 Human Na_v1.8 (SCN10A)

Cloned cDNA sequence of human Na_v1.8 subunit was codon optimized and encodes for Q9Y5Y9.2.

3.2 Human Na_v1.8 Beta 3 (SCN3B)

The Beta Subunit SCN3B was cotransfected to increase surface expression of the Alpha Subunit SCN10A. the cDNA was codon optimized and encodes for NM_060870.1.

4. CONTACT INFORMATION

4.1 Contact Address for Technical Support & Ordering Information

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