B'SYS GmbH

CHO $Na_V1.5$ Cell Line

Specification Sheet

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1 BACKGROUND

1.1 The cardiac I_{Na} is encoded by $Na_V1.5$

Around 20 different ionic currents can be divided into depolarizing and repolarizing currents involved in the generation of the cardiac action potential. The rapid depolarizing of the cell membrane at the beginning of the cardiac action potential is due to the activation of voltage-dependent sodium $Na_V1.5$ channels.

1.2 The Long QT Syndrome 3

Mutations in the SCNA5 gene result in persistent depolarizing current throughout action potential duration causing the Long QT Syndrome 3 phenotype

1.3 B'SYS' CHO $Na_V1.5$ cells

B'SYS has designed a new CHO $Na_V1.5$ cell line with constitutive expression of human $Na_V1.5$ sodium channels. The human $Na_V1.5$ cDNA was cloned and transfected into CHO cells and then the functional properties of the $Na_V1.5$ channels validated by means of automated patch-clamp technique (Q-Patch). Results and success rates are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human $Na_V1.5$ channel:

1 x 0.5 mL aliquots of frozen cells at 2.2 E+06 cells/mL
Cells are frozen in complete medium with 10% DMSO

2.2 Mycoplasma Certificate

B'SYS periodically tests cells for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

3 VALIDATION OF CHO NAV1.5 CELLS

3.1 Biophysical characterization

Nav1.5 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 GÙ seal between the patch electrodes and individual Nav1.5 stably transfected CHO 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 to -130 mV up to +20 mV in 10 mV increments of 300 ms duration. Tail currents are elicited upon subsequent repolarization of the cell membrane to -10 mV for 30 ms. (Fig. 1 A, B). The voltage pulses were run at intervals of 5 s.

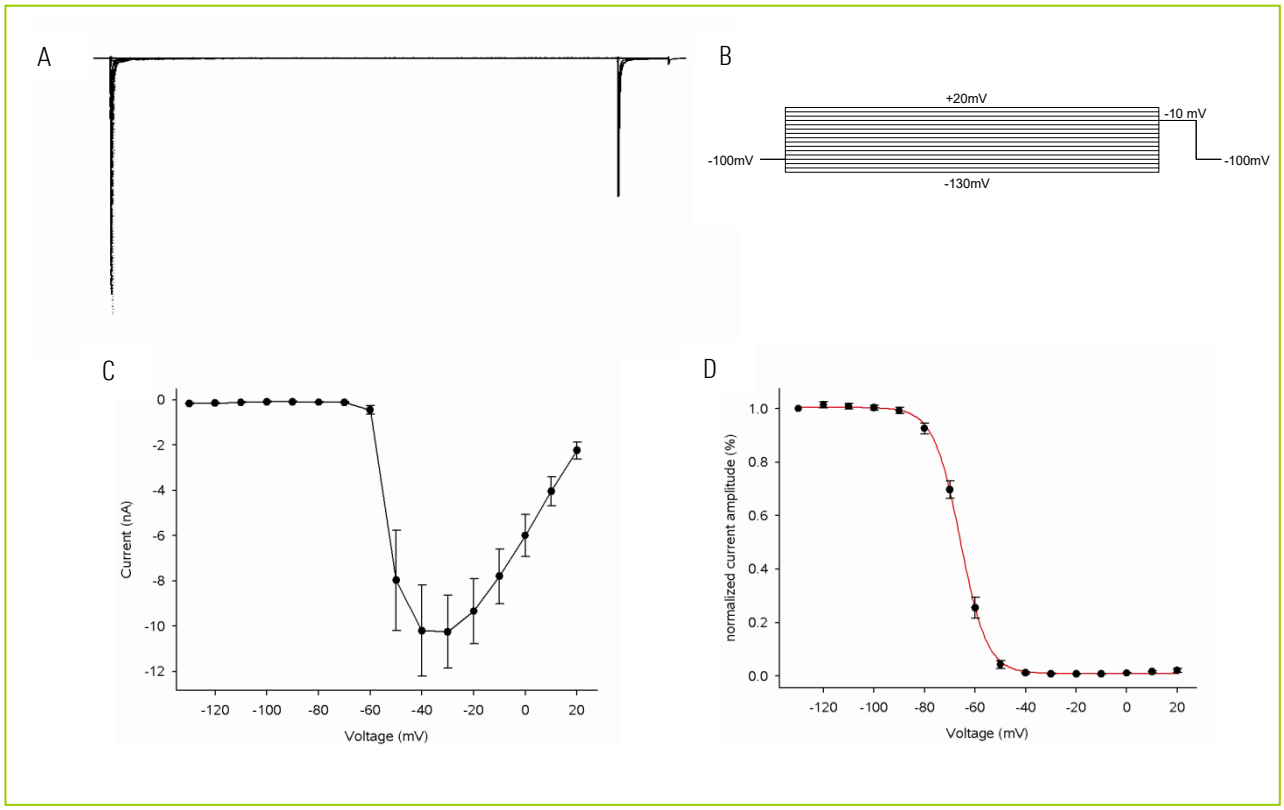

Fig. 1: Biophysical Characterization of stably transfected CHO Nav1.5 cells. A) representative current recording. Cells were stimulated by the voltage protocol shown in b). C) IV curve if the Nav1.5 channel. Peak current were measured at the beginning of the voltage pulse (n=11). D) Inactivation curve of the Nav1.5 channel. The current amplitudes were measured at the beginning of the tail current. Data were fit by a Boltzmann equation. The $V_{0.5}$ value was 65.83 ± 0.88 mV (n=11). No currents were recorded in untransfected cells (data not shown).

For the IV curve the peak current amplitude of the pulses between -130 mV and +20 mV was plotted versus the applied voltage. This resulted in a bell shaped curve. The minimum of the IV curve was between 30 mV and -40 mV (Fig 1 C).
For the inactivation curve the peak currents of the tail current (pulse to -10 mV) was plotted versus the voltage of the prepulse. The resulting inactivation curve was fit with a Boltzmann equation. The $V_{0.5}$ value was 65.83 ± 0.88 mV (n=11), (Fig 1 D). This validation shows the same biophysical characteristics like described in the literature (Persson et al. 2005 and Mantegazza et al. 2001).

3.2 Pharmacological characterization

Nav1.5 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 / intracellular solution consisted of (in mM) CsF 135 mM, NaCl 10 mM, HEPES 10 mM, EGTA 5 mM, pH (KOH) 7.3.
For the pharmacological characterization of the Nav1.5 channel Propafenone was tested at concentrations of 0.3, 1.0, 3.0 and 10 µM, Quinidine at concentrations of 1.0, 3.0, 10 and 30 µM (n≥3 cells) at stimulation frequencies of 1 or 3 Hz. The dose response curves were generated (see Fig. 2) and the IC_{50} values calculated.
The Nav1.5 currents were stimulated by a 50 ms pulse to -10 mV from a holding potential of -100 mV.

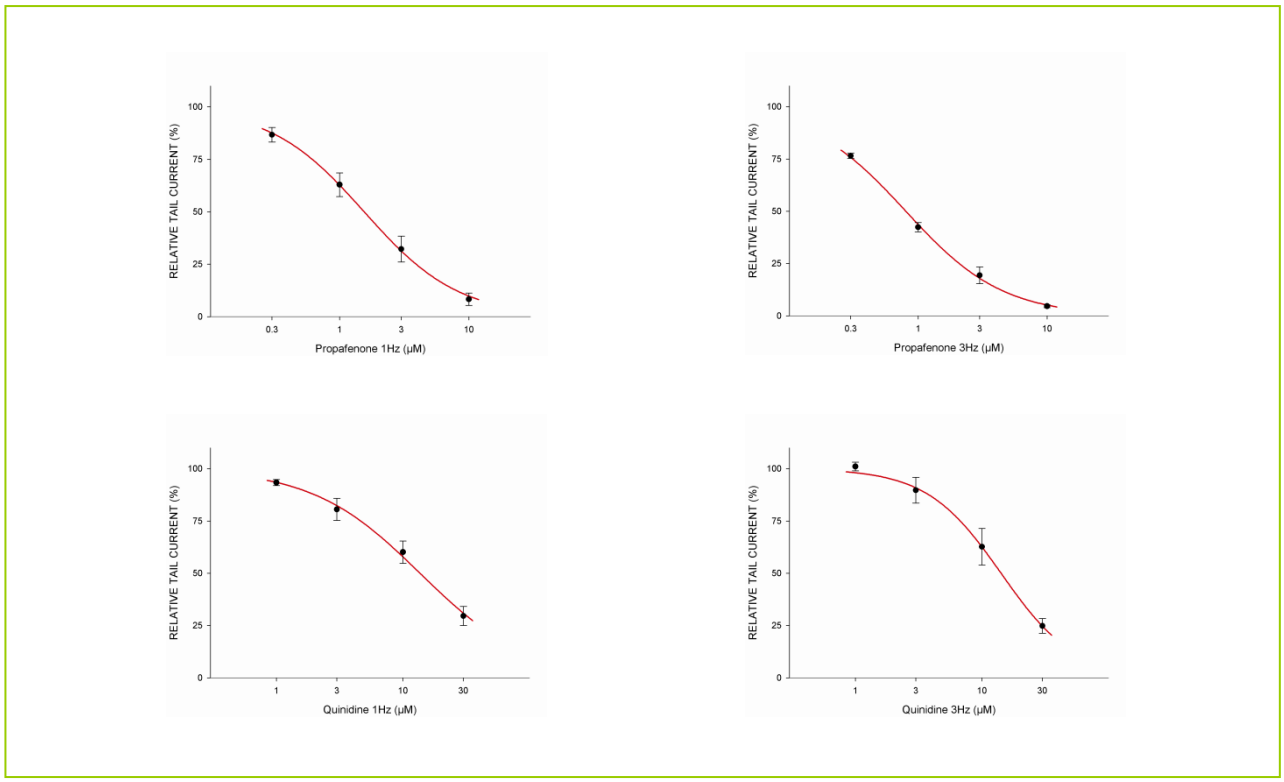

Fig. 2: Dose response curves for Propafenone and Quinidine at stimulation frequencies of 1 and 3Hz.

The results can be summarized as follows.

Application / stimulation frequency	IC_{50} value (µM)	Hill coefficient
Propafenone / 1Hz	1.55	1.19
Propafenone / 3Hz	0.80	1.15
Quinidine / 1Hz	13.64	1.02
Quinidine / 3Hz	14.15	1.48

For the pharmacological characterization of the Nav1.5 channel using automated patch-clamping, Hydroxychloroquine was tested at concentrations of 3, 10, 30, 100 and 300 µM, at stimulation frequency of 0.1 Hz. The dose response curves were generated (see Fig. 3) and the IC_{50} value was calculated to be 44.15 µM, Hill coefficient: 0.85.
The Nav1.5 currents were stimulated by a 40 ms pulse to -15 mV from a holding potential of -120 mV (using the CiPA voltage protocol).

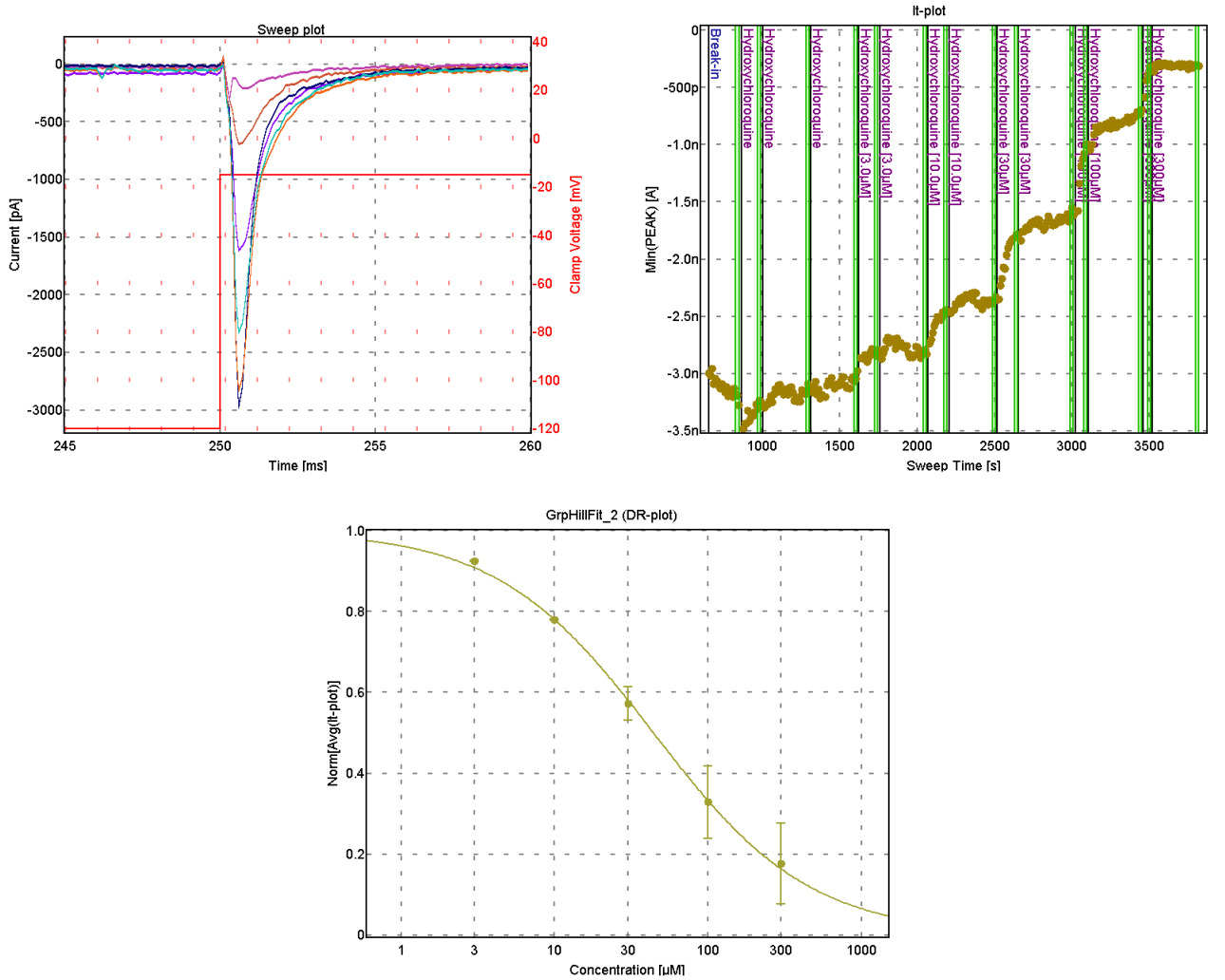

Fig. 3: Effects of Hydroxychloroquine on Nav1.5 peak current amplitudes: Top left: representative current recording, top right: IT plot of one cell treated with increasing concentrations of Hydroxychloroquine, bottom: Dose response curve for Hydroxychloroquine.

3.3 Patch-clamp Success Rates

The patch-clamp properties of the CHO Nav1.5 cell line were elucidated using automated patch-clamping (Q.-Patch). A total of 120 cells were analyzed:

	Total tested wells	Cells attached	Giga ohm seal	Whole cell	Completed experiments
Number of cells	120	117	112	115	104
Success rate	-	97.5%	93.3%	95.8%	86.6%

4 CELL CULTURE CONDITIONS

4.1 General

CHO $Na_V1.5$ cells are incubated at 37°C in a humidified atmosphere with 5% CO_2 (rel. humidity > 95%). The cells are continuously maintained and passaged in sterile culture flasks containing F12 (HAM) medium supplemented with 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and 7 µg/mL Puromycin and 5 µg/mL Blasticidin. The CHO $Na_V1.5$ cells are passaged at a confluence of about 80%. For manual electrophysiological measurements the cells are seeded onto e.g. 35 mm sterile culture dishes, for automated patch clamping on T25 cell culture flasks containing complete medium.

All solutions and equipment coming in contact with the cells must be sterile.
Use proper sterile technique and work in a laminar flow hood.
Be sure to have frozen cell stocks at hand before starting experiments.
Cells should be split every 2-3 days at 70% - 80% confluency at 1:3 to 1:5 ratio.

4.2 Recommended Complete Medium

- 500 mL F12 (HAM) with L-Glutamine or GlutaMax I
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Selection antibiotics

CHO $Na_V1.5$ clones were selected under 10 µg/mL Puromycin and 5 µM Blasticidin antibiotic pressure.
To cultivate CHO $Na_V1.5$ cells, a antibiotic concentration of 7 µg/mL Puromycin and 5 µM Blasticidin is recommeded.

Remark: The permanent application of high antibiotic pressure has no effect on current density.

4.4 Thawing Cells

Remove vial of cells from liquid nitrogen and thaw quickly at 37°C.
Decontaminate outside of vial with 70% Ethanol.
Transfer cells to a T-75 culture flask containing 10 mL complete medium.
Incubate cells at 37°C for 4-6 h to allow the cells to attach to the bottom of flask.
Aspirate off the medium and replace with 10 mL complete medium and selection antibiotics.
Incubate cells and check them daily until 70% - 80% confluency is reached.

4.5 Splitting Cells

When cells are 70% - 80% confluent remove medium.
Wash cells once with 1x PBS to remove excess medium.
Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
Remove Trypsin/EDTA quickly and incubate cells for 2 min at 37°C.
Detach cells, add complete medium and pipet up and down to break clumps of cells.
Passage cells into new flask with complete medium and antibiotics at 1:3 to 1:5 ratio.
Use remaining suspension for counting the cells.

4.6 Freezing Medium

Mix 0.9 mL fresh complete medium and 0.1 mL DMSO for every 1 mL freezing medium.
Use sterile DMSO or sterilize freezing medium by means of appropriate micro filter (0.1 µm – 0.2 µm).

4.7 Freezing Cells

Prepare fresh freezing medium and keep it on ice.
Cells should have 80% - 90% confluency prior to freezing.
Remove the complete medium.
Wash cells once with 1x PBS to remove excess medium.
Add 1x Trypsin/EDTA and incubate 30 s at room temperature.
Remove Trypsin/EDTA quickly and incubate cells for 2 min at 37°C.
Detach cells, add complete medium and pipet up and down to break clumps of cells.
Pellet cells with centrifuge and carefully aspirate off medium.
Resuspend cells at a density of approximately 2.0 E+06 cells per mL with fresh freezing medium.
Aliquot 0.5 mL of cell suspension into each cryovial.
Overnight incubate cells in a styropor box at –80°C.
The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

5 Nav1.5 SEQUENCE

5.1 Human Nav1.5 Accession Number NM_000335

Cloned cDNA sequence of human Nav1.5 subunit was error-free and identical with NM_000335 sequence encoding for NP_000326:

MANFLLPRGTSSFRRFTRESLAAIEKRMAEKQARGSTTLQESREGLPEEEAPRPQLDLQASKKLPDLYGNPPQELIGEPL
EDLDPFYSTQKTFIVLNKGKTIFRFSATNALYVLSPFHPIRRAAVKILVHSLFNMLIMCTILTNCVFMAQHDPPPWTKYV
EYTFTAIYTFESLVKILARGFCLHAFTFLRDPWNWLDFSVIIMAYTTEFVDLGNVSALRTFRVLRALKTISVISGLKTIV
GALIQSVKKLADVMVLTVFCLSVFALIGLQLFMGNLRHKCVRNFTALNGTNGSVEADGLVWESLDLYLSDPENYLLKNGT
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MMDFFHAFLIIFRILCGEWIETMWDCMEVSGQSLCLLVFLLVMVIGNLVVLNLFLALLLSSFSADNLTAPDEDREMNNLQ
LALARIQRGLRFVKRTTWDFCCGLLRQRPQKPAALAAQGQLPSCIATPYSPPPPETEKVPPTRKETRFEEGEQPGQGTPG
DPEPVCVPIAVAESDTDDQEEDEENSLGTEEESSKQESQPVSGGPEAPPDSRTWSQVSATASSEAEASASQADWRQQWKA
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IVVNMYIAIILENFSVATEESTEPLSEDDFDMFYEIWEKFDPEATQFIEYSVLSDFADALSEPLRIAKPNQISLINMDLP
MVSGDRIHCMDILFAFTKRVLGESGEMDALKIQMEEKFMAANPSKISYEPITTTLRRKHEEVSAMVIQRAFRRHLLQRSL
KHASFLFRQQAGSGLSEEDAPEREGLIAYVMSENFSRPLGPPSSSSISSTSFPPSYDSVTRATSDNLQVRGSDYSHSEDL
ADFPPSPDRDRESIV–

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