B'SYS GmbH

CHO $Na_V1.8/\beta_3$ Cell Line

Specification Sheet

TABLE OF CONTENTS

1 BACKGROUND

1.1 B'SYS' CHO $Na_V1.8/\beta_3$ cells

B'SYS has designed a CHO $Na_V1.8/\beta_3$ cell line. The human $Na_V1.8$ (SCN10A) and the β_3 subunit (SCN3B) cDNA was cloned and transfected into CHO cells and then the functional properties of the $Na_V1.8/\beta_3$ channels validated by means of the patch-clamp technique. Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

CHO cells stably transfected with recombinant human $Na_V1.8/\beta_3$ channel:

- 1 x 0.5 mL aliquots of frozen cells at 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 NAV1.8/B3 CELLS

3.1 Pharmacological and biophysical characterization

$Na_V1.8/\beta_3$ 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, $CaCl_2$ 1.8 mM, $MgCl_2$ 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, $MgCl_2$ 6 mM, HEPES 10 mM, EGTA 5 mM, pH (KOH) 7.2. After formation of a Gigaohm seal between the patch electrodes and individual $Na_V1.5$ 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 -90 mV to +10 mV in 6 s intervals. 100 µM Tetracaine blocked the sodium inward current efficiently (Fig. 1 A).
To determine the voltage dependence of inactivation, cells were clamped form a holding potential of -90 mV to -120 mV up to 0 mV in 10 mV increments of 500 ms duration. Tail currents are elicited upon subsequent repolarization of the cell membrane to 0 mV for 50 ms. (Fig. 1 B). The voltage pulses were run at intervals of 6 ms: $V_{0.5}$= -72.52 ± 1.71 mV, k= -9.29 ± 0.66 (n=12)

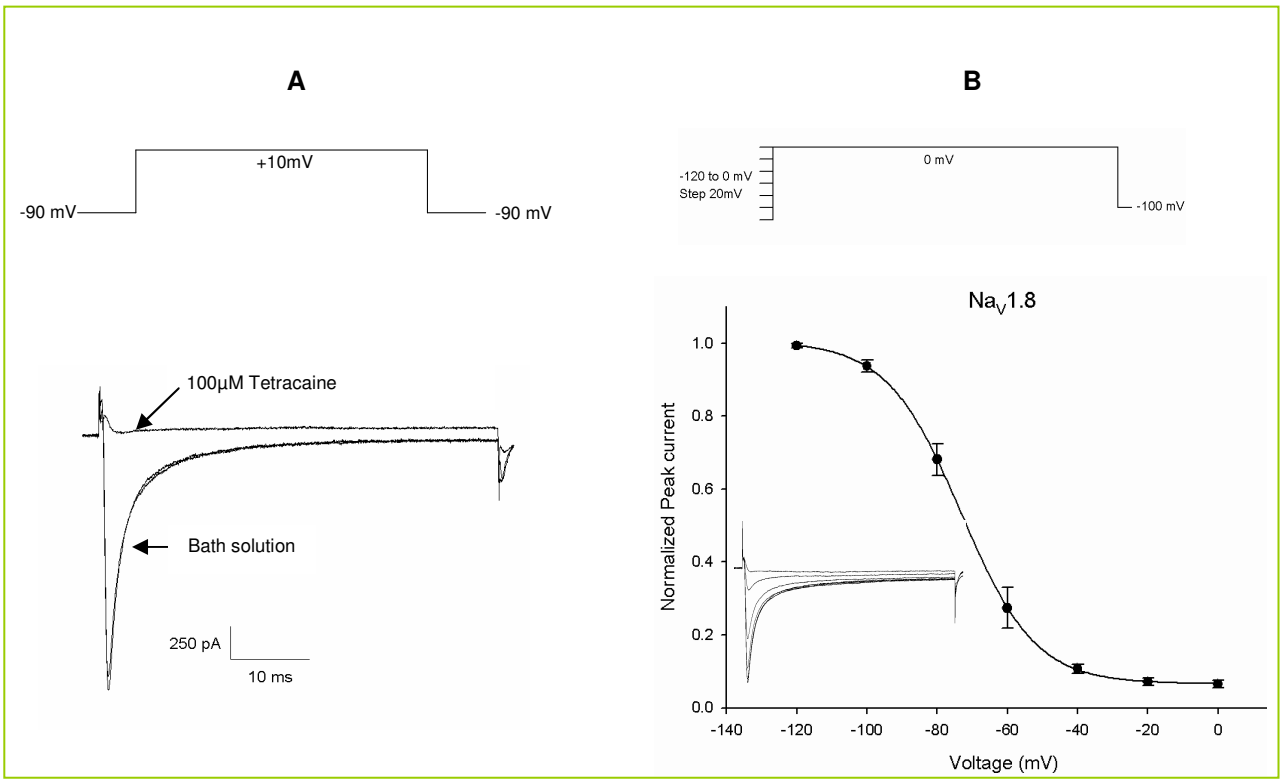


Fig. 1: A) $Na_V1.8/\beta_3$ currents in control conditions and after application of 100 µM Tetracaine. B) Inactivation curve of $Na_V1.8$ channels and corresponding representative current recordings.

3.2 Pharmacological characterization

For the pharmacological characterization of the $Na_V1.8/\beta_3$ channel Tetracaine was tested at concentrations of 0.3, 1.0, 3.0, 10, 30 and 100 µM the IC_{50} value calculated.
The $Na_V1.8/\beta_3$ currents were stimulated by a 50 ms pulse to 0 mV from a holding potential of -90 mV.

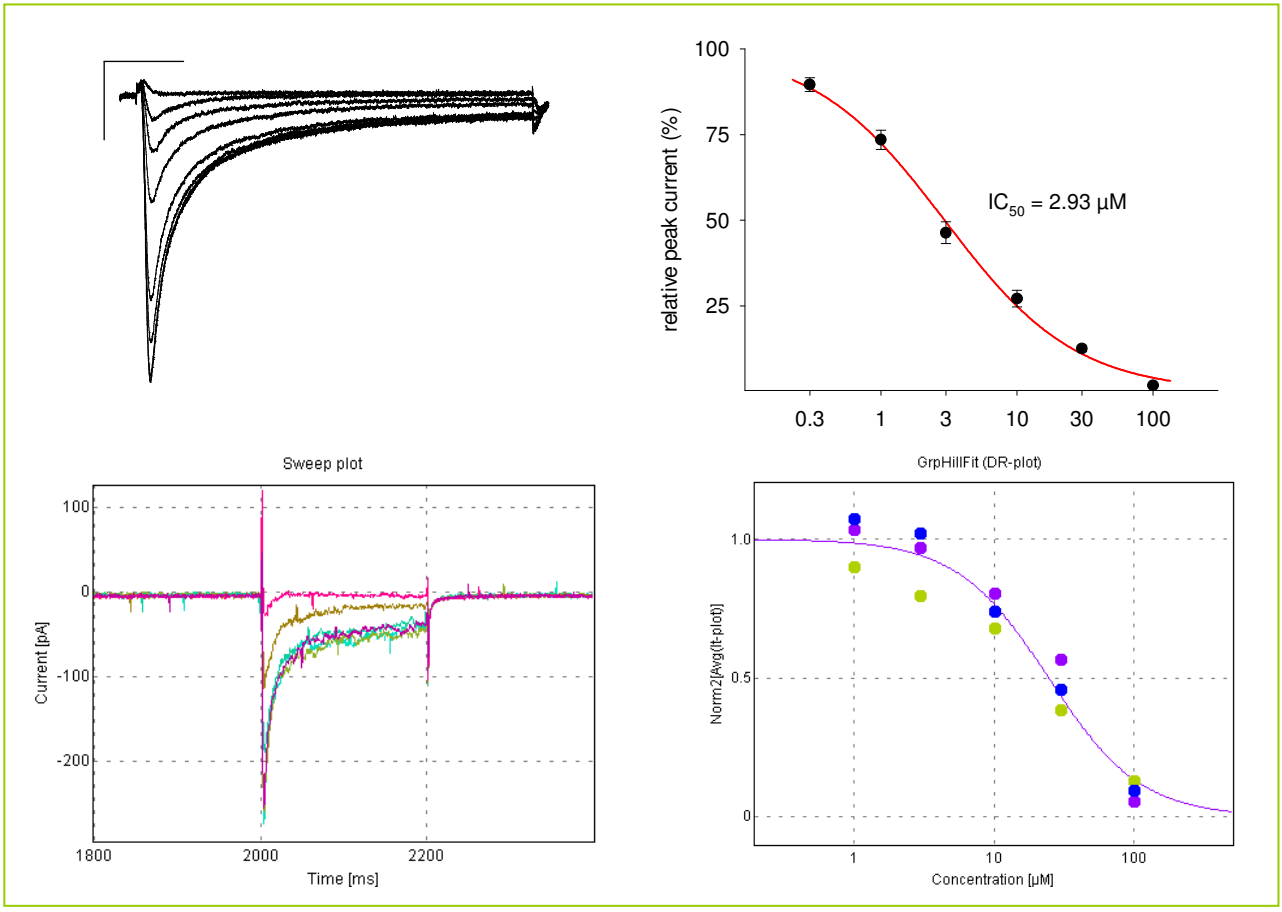


Fig. 2: Dose response curves for Tetracaine. Top: manual patch-clamping. left: representative current recording. scale bars: 500 pA, 50 ms, right: dose response curve. Bottom: automated patch-clamping. left: representative current recording, right: dose response curve.

Compound	IC_{50} value	
	Manual patch-clamping	Automated patch-clamping
Terfenadine	2.93 µM	24.42 µM
A-803467	41.37 nM	

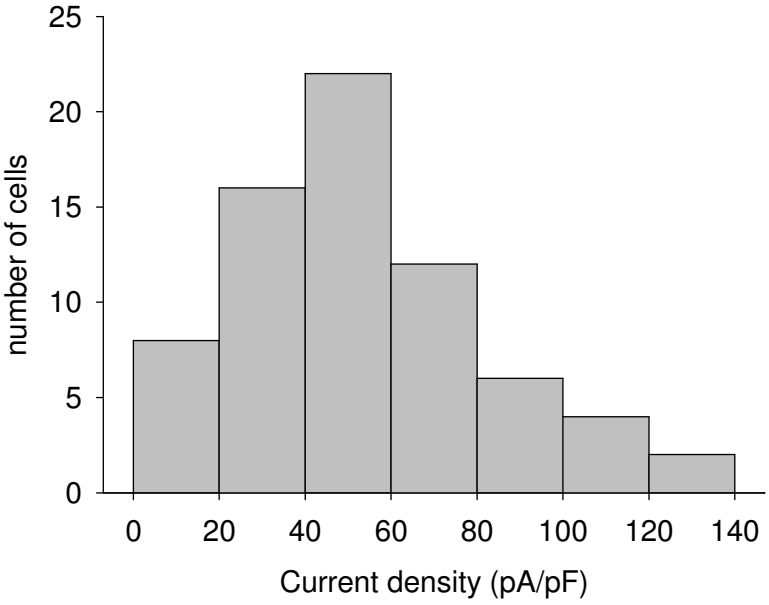
3.3 Patch-clamp Success Rates and Current Density

The patch-clamp properties of the CHO $Na_V1.8/\beta_3$ cell line were elucidated at typical working passage numbers (passage 5-29).

Success Rates for automated patch-clamping (Q-Patch):

- Whole-cell successful: **75%** (n=94)
- Cells with $Na_V1.8/\beta_3$ currents: **80%** (n=70)
- Recording (15 min) successful: **70%** (n=70)

Distribution of current density (n=70):



4 CELL CULTURE CONDITIONS

4.1 General

CHO $Na_V1.8/\beta_3$ 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 350 µg/mL Hygromycin and 3.5 µg/mL Puromycin. The CHO $Na_V1.8/\beta_3$ 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 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

- F12 (HAM) with Glutamine or GlutaMAX I
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Antibiotics

- CHO $Na_V1.8/\beta_3$ clones were selected under 500 µg/mL Hygromycin and 5.0 µg/mL Puromycin antibiotic pressure.
- To cultivate CHO $Na_V1.8/\beta_3$ cells, also a reduced antibiotic pressure (350 µg/mL Hygromycin and 3.5 µg/mL Puromycin) can be used.

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-25 culture flask containing 5 mL complete medium without antibiotics.
- 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 5 mL complete medium & antibiotics.
- Antibiotics: 350 µg/mL Hygromycin and 3.5 µg/mL Puromycin.
- 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.
- 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 NAV1.8/B3 SEQUENCE

5.1 Human SCN10A

Cloned cDNA sequence of human SCN10A subunit was error-free and encoded for NP_006505 sequence:

MEFPIGSLETNNFRRFTPESLVEIEKQIAAKQGTKKAREKHREQKDQEEKPRPQLDLKACNQLPKFYGEL
PAELIGEPLEDLDPFYSTHRTFMVLNKGRTISRFSATRALWLFSPFNLIRRTAIKVSVHSWFSLFITVTI
LVNCVCMTRTDLPEKIEYVFTVIYTFEALIKILARGFCLNEFTYLRDPWNWLDFSVITLAYVGTAIDLRG
ISGLRTFRVLRALKTVSVIPGLKVIVGALIHSVKKLADVTILTIFCLSVFALVGLQLFKGNLKNKCVKND
MAVNETTNYSSHRKPDIYINKRGTSDPLLCGNGSDSGHCPDGYICLKTSDNPDFNYTSFDSFAWAFLSLF
RLMTQDSWERLYQQTLRTSGKIYMIFFVLVIFLGSFYLVNLILAVVTMAYEEQNQATTDEIEAKEKKFQE
ALEMLRKEQEVLAALGIDTTSLHSHNGSPLTSKNASERRHRIKPRVSEGSTEDNKSPRSDPYNQRRMSFL
GLASGKRRASHGSVFHFRSPGRDISLPEGVTDDGVFPGDHESHRGSLLLGGGAGQQGPLPRSPLPQPSNP
DSRHGEDEHQPPPTSELAPGAVDVSAFDAGQKKTFLSAEYLDEPFRAQRAMSVVSIITSVLEELEESEQK
CPPCLTSLSQKYLIWDCCPMWVKLKTILFGLVTDPFAELTITLCIVVNTIFMAMEHHGMSPTFEAMLQIG
NIVFTIFFTAEMVFKIIAFDPYYYFQKKWNIFDCIIVTVSLLELGVAKKGSLSVLRSFRLLRVFKLAKSW
PTLNTLIKIIGNSVGALGNLTIILAIIVFVFALVGKQLLGENYRNNRKNISAPHEDWPRWHMHDFFHSFL
IVFRILCGEWIENMWACMEVGQKSICLILFLTVMVLGNLVVLNLFIALLLNSFSADNLTAPEDDGEVNNL
QVALARIQVFGHRTKQALCSFFSRSCPFPQPKAEPELVVKLPLSSSKAENHIAANTARGSSGGLQAPRGP
RDEHSDFIANPTVWVSVPIAEGESDLDDLEDDGGEDAQSFQQEVIPKGQQEQLQQVERCGDHLTPRSPGT
GTSSEDLAPSLGETWKDESVPQVPAEGVDDTSSSEGSTVDCLDPEEILRKIPELADDLEEPDDCFTEGCI
RHCPCCKLDTTKSPWDVGWQVRKTCYRIVEHSWFESFIIFMILLSSGSLAFEDYYLDQKPTVKALLEYTD
RVFTFIFVFEMLLKWVAYGFKKYFTNAWCWLDFLIVNISLISLTAKILEYSEVAPIKALRTLRALRPLRA
LSRFEGMRVVVDALVGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGKFWRCINYTDGEFSLVPLSIVNNKS
DCKIQNSTGSFFWVNVKVNFDNVAMGYLALLQVATFKGWMDIMYAAVDSREVNMQPKWEDNVYMYLYFVI
FIIFGGFFTLNLFVGVIIDNFNQQKKKLGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPLNKFQGFVF
DIVTRQAFDITIMVLICLNMITMMVETDDQSEEKTKILGKINQFFVAVFTGECVMKMFALRQYYFTNGWN
VFDFIVVVLSIASLIFSAILKSLQSYFSPTLFRVIRLARIGRILRLIRAAKGIRTLLFALMMSLPALFNI
GLLLFLVMFIYSIFGMSSFPHVRWEAGIDDMFNFQTFANSMLCLFQITTSAGWDGLLSPILNTGPPYCDP
NLPNSNGTRGDCGSPAVGIIFFTTYIIISFLIVVNMYIAVILENFNVATEESTEPLSEDDFDMFYETWEK
FDPEATQFITFSALSDFADTLSGPLRIPKPNRNILIQMDLPLVPGDKIHCLDILFAFTKNVLGESGELDS
LKANMEEKFMATNLSKSSYEPIATTLRWKQEDISATVIQKAYRSYVLHRSMALSNTPCVPRAEEEAASLP
DEGFVAFTANENCVLPDKSETASATSFPPSYESVTRGLSDRVNMRTSSSIQNEDEATSMELIAPGP

5.2 Human SCN3B

Cloned cDNA sequence of human SCN3B subunit was error-free and encoded for NP_060870 sequence:

MPAFNRLFPLASLVLIYWVSVCFPVCVEVPSETEAVQGNPMKLRCISCMKREEVEATTVVEWFYRPEGGK
DFLIYEYRNGHQEVESPFQGRLQWNGSKDLQDVSITVLNVTLNDSGLYTCNVSREFEFEAHRPFVKTTRL
IPLRVTEEAGEDFTSVVSEIMMYILLVFLTLWLLIEMIYCYRKVSKAEEAAQENASDYLAIPSENKENSA
VPVEE

6 CONTACT INFORMATION

6.1 Contact Address for Technical Support & Ordering Information

- B'SYS GmbH
  Technology Center Witterswil
  Benkenstrasse 254
  4108 Witterswil
  Switzerland

  Tel: +41 61 721 77 44
  Fax: +41 61 721 77 41
  Email: info@bsys.ch
  Web: www.bsys.ch