B'SYS GmbH

HEK $Ca_V3.2$ Cell Line

Specification Sheet

TABLE OF CONTENTS

1 BACKGROUND

1.1 B'SYS's HEK $Ca_V3.2$ Cells

B'SYS has designed a HEK $Ca_V3.2$ cell line. The human $Ca_V3.2$ cDNA was cloned and transfected into HEK cells and then the functional properties of the $Ca_V3.2$ channels validated by means of the patch-clamp technique. Results are outlined in section 3.

2 PRODUCT SHIPMENT

2.1 Product Format

HEK cells stably transfected with recombinant human $Ca_V3.2$ channel:

- 2 x 0.5 mL aliquots of frozen cells at approximately 1.0 - 3.0 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 HEK $CA_V3.2$ CELLS

3.1 Electrophysiology

$Ca_V3.2$ currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. After formation of a GΩ seal between the patch electrodes and individual $Ca_V3.2$ stably transfected HEK 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 inward calcium currents were measured upon depolarization of the cell membrane from a holding potential of -80 mV to +60 mV in 10 mV increments of 50 ms duration (Fig. 1). The voltage pulses were run at intervals of 10 s.

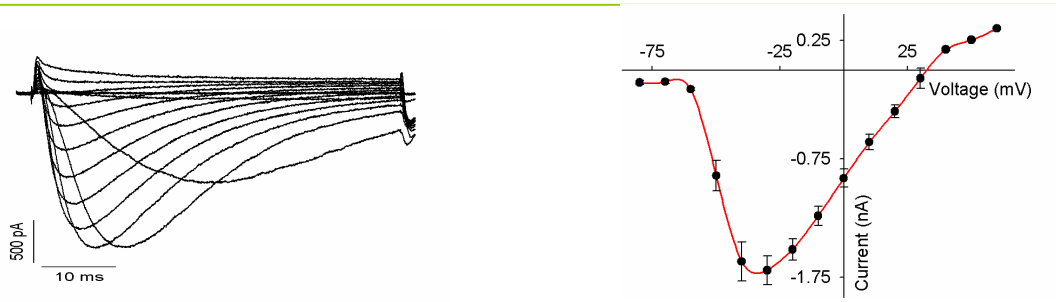

Fig.1: Representative current recording and IV curve of $Ca_V3.2$ currents upon depolarization of the cell membrane from holding potential (-80 mV) up to +60 mV in 10 mV increments. No inward calcium currents were recorded in untransfected cells (data not shown).

3.2 Positive Control Mibefradil

Mibefradil, which is known as a T-Type Ca_V blocker, was used as positive control to validate the pharmacological profile of the HEK $Ca_V3.2$ cells. The effect of Mibefradil was first measured at 10 µM, which blocked $Ca_V3.2$ currents almost completely (9.1 ± 2.3 % relative current amplitude, mean ± SEM of 5 cells). When the cells were exposed to 0.01, 0.1, 1.0 and 10 µM Mibefradil, the steady-state level relative current amplitudes revealed values of 84.7 ± 3.2%, 57.3 ± 4.0%, 19.1 ± 2.6 and 9.1 ± 2.3%, respectively (mean ± SEM of 3 cells). The inhibition curve (Fig. 2) was best fitted with an IC_{50} value of **143.7 nM**.

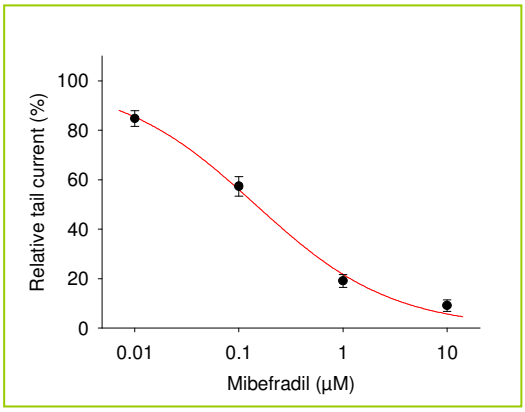

Fig. 2: $Ca_V3.2$ current inhibition curve of Mibefradil.

3.3 Patch-clamp Success Rates

The patch-clamp properties of the HEK $Ca_V3.2$ cell line were elucidated at typical working passage numbers. A total of 17 cells were analyzed. Success for establishment of on-cell configuration was defined as follows: > 1 GΩ. The whole-cell configuration was not accepted if the membrane resistance was below 1 GΩ. A successful recording had to be free of rundown effects and variations in series resistance.

- On-cell successful: **91%** (n=17)
- Whole-cell successful: **82%** (n=17)
- Recording successful: **73%** (n=17)

4 CELL CULTURE CONDITIONS

4.1 General

HEK $Ca_V3.2$ 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 DMEM/F12 medium supplemented with 10% fetal bovine serum, 1.0% Penicillin/Streptomycin solution and 100 µg/mL G-418. The HEK $Ca_V3.2$ cells are passaged at a confluence of about 50-80%. For 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 splitted every 4-5 days at 50% - 80% confluency at 1:3 to 1:5 ratio.

4.2 Recommended Complete Medium

- DMEM/F12 with GlutaMAX I or L-Glutamine
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Antibiotics

- HEK $Ca_V3.2$ clones were selected under 500 µg/mL G-418 antibiotic pressure.
- To cultivate HEK $Ca_V3.2$ cells, a reduced antibiotic pressure (100 µg/mL) should be used.

Remark: The permanent application of 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.
- 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: 100 µg/ml G-418.
- Incubate cells and check them daily until 50% - 80% confluency is reached.

4.5 Splitting Cells

- When cells are 50% - 80% confluent remove complete medium.
- Wash cells once with 1x Trypsin/EDTA.
- Remove Trypsin/EDTA quickly and incubate cells for 3-5 min at room temperature.
- 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 Trypsin/EDTA.
- Remove Trypsin/EDTA quickly and incubate cells for 3-5 min at room temperature.
- 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 3.0 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 $CA_V3.2$ SEQUENCE

5.1 $Ca_V3.2$

Cloned cDNA sequence of $Ca_V3.2$ (CACNA1H) subunit was error-free and is encoding for NP_066921:

MTEGARAADEVRVPLGAPPPGPAALVGASPESPGAPGREAERGSELGVSPSESPAAERGAELGADEEQRVPYPAL
AATVFFCLGQTTRPRSWCLRLVCNPWFEHVSMLVIMLNCVTLGMFRPCEDVECGSERCNILEAFDAFIFAFFAVE
MVIKMVALGLFGQKCYLGDTWNRLDFFIVVAGMMEYSLDGHNVSLSAIRTVRVLRPLRAINRVPSMRILVTLLLD
TLPMLGNVLLLCFFVFFIFGIVGVQLWAGLLRNRCFLDSAFVRNNNLTFLRPYYQTEEGEENPFICSSRRDNGMQ
KCSHIPGRRELRMPCTLGWEAYTQPQAEGVGAARNACINWNQYYNVCRSGDSNPHNGAINFDNIGYAWIAIFQVI
TLEGWVDIMYYVMDAHSFYNFIYFILLIIVGSFFMINLCLVVIATQFSETKQRESQLMREQRARHLSNDSTLASF
SEPGSCYEELLKYVGHIFRKVKRRSLRLYARWQSRWRKKVDPSAVQGQGPGHRQRRAGRHTASVHHLVYHHHHHH
HHHYHFSHGSPRRPGPEPGACDTRLVRAGAPPSPPSPGRGPPDAESVHSIYHADCHIEGPQERARVAHAAATAAA
SLRLATGLGTMNYPTILPSGVGSGKGSTSPGPKGKWAGGPPGTGGHGPLSLNSPDPYEKIPHVVGEHGLGQAPGH
LSGLSVPCPLPSPPAGTLTCELKSCPYCTRALEDPEGELSGSESGDSDGRGVYEFTQDVRHGDRWDPTRPPRATD
TPGPGPGSPQRRAQQRAAPGEPGWMGRLWVTFSGKLRRIVDSKYFSRGIMMAILVNTLSMGVEYHEQPEELTNAL
EISNIVFTSMFALEMLLKLLACGPLGYIRNPYNIFDGIIVVISVWEIVGQADGGLSVLRTFRLLRVLKLVRFLPA
LRRQLVVLVKTMDNVATFCTLLMLFIFIFSILGMHLFGCKFSLKTDTGDTVPDRKNFDSLLWAIVTVFQILTQED
WNVVLYNGMASTSSWAALYFVALMTFGNYVLFNLLVAILVEGFQAEGDANRSDTDEDKTSVHFEEDFHKLRELQT
TELKMCSLAVTPNGHLEGRGSLSPPLIMCTAATPMPTPKSSPFLDAAPSLPDSRRGSSSSGDPPLGDQKPPASLR
SSPCAPWGPSGAWSSRRSSWSSLGRAPSLKRRGQCGERESLLSGEGKGSTDDEAEDGRAAPGPRATPLRRAESLD
PRPLRPAALPPTKCRDRDGQVVALPSDFFLRIDSHREDAAELDDDSEDSCCLRLHKVLEPYKPQWCRSREAWALY
LFSPQNRFRVSCQKVITHKMFDHVVLVFIFLNCVTIALERPDIDPGSTERVFLSVSNYIFTAIFVAEMMVKVVAL
GLLSGEHAYLQSSWNLLDGLLVLVSLVDIVVAMASAGGAKILGVLRVLRLLRTLRPLRVISRAPGLKLVVETLIS
SLRPIGNIVLICCAFFIIFGILGVQLFKGKFYYCEGPDTRNISTKAQCRAAHYRWVRRKYNFDNLGQALMSLFVL
SSKDGWVNIMYDGLDAVGVDQQPVQNHNPWMLLYFISFLLIVSFFVLNMFVGVVVENFHKCRQHQEAEEARRREE
KRLRRLERRRRSTFPSPEAQRRPYYADYSPTRRSIHSLCTSHYLDLFITFIICVNVITMSMEHYNQPKSLDEALK
YCNYVFTIVFVFEAALKLVAFGFRRFFKDRWNQLDLAIVLLSLMGITLEEIEMSAALPINPTIIRIMRVLRIARV
LKLLKMATGMRALLDTVVQALPQVGNLGLLFMLLFFIYAALGVELFGRLECSEDNPCEGLSRHATFSNFGMAFLT
LFRVSTGDNWNGIMKDTLRECSREDKHCLSYLPALSPVYFVTFVLVAQFVLVNVVVAVLMKHLEESNKEAREDAE
LDAEIELEMAQGPGSARRVDADRPPLPQESPGARDAPNLVARKVSVSRMLSLPNDSYMFRPVVPASAPHPRPLQE
VEMETYGAGTPLGSVASVHSPPAESCASLQIPLAVSSPARSGEPLHALSPRGTARSPSLSRLLCRQEAVHTDSLE
GKIDSPRDTLDPAEPGEKTPVRPVTQGGSLQSPPRSPRPASVRTRKHTFGQRCVSSRPAAPGGEEAEASDPADEE
VSHITSSACPWQPTAEPHGPEASPVAGGERDLRRLYSVDAQGFLDKPGRADEQWRPSAELGSGEPGEAKAWGPEA
EPALGARRKKKMSPPCISVEPPAEDEGSARPSAAEGGSTTLRRRTPSCEATPHRDSLEPTEGSGAGGDPAAKGER
WGQASCRAEHLTVPSFAFEPLDLGVPSGDPFLDGSHSVTPESRASSSGAIVPLEPPESEPPMPVGDPPEKRRGLY
LTVPQCPLEKPGSPSATPAPGGGADDPV

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