B'SYS GmbH

CHO $Na_V1.5$ Duo New

Cell culture conditions

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1 PRODUCT SHIPMENT

1.1 Product Format

CHO cells stably transfected with recombinant human Nav1.5 sodium channel:

- 0.75 mL aliquots of frozen cells at approximately 3.0 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO

1.2 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.

2 CELL CULTURE CONDITIONS

2.1 General

CHO Nav1.5 sodium channel 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 15 µg/mL blasticidin and 100 µg/mL zeocin. The CHO Nav1.5 sodium channel cells are passaged at a confluence of about 60 to 80%.

To increase Nav1.5-mediated currents, 2.5 µg/mL Tetracycline can be added 24-48 h before experimentation.

- 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% to 80% confluency at 1:3 to 1:5 ratio.

Table 1: Cell culture reagents

Product	Supplier	Order number
Nutrient mixture F-12 Ham (F12 Ham)	Sigma-Aldrich	N6658
Fetal Bovine Serum (FBS)	Gibco	10270-106
Penicillin / Streptomycin (100x)	Gibco	10378-016
Phosphate Buffered Saline (PBS, without Ca^{2+} and Mg^{2+})	Sigma-Aldrich	D8537
Blasticidin S HCl	Gibco	R21001
Zeocin (100 mg/mL)	Gibco	R25001
Detachin	Genlantis	T100T100
Trypsin EDTA (10x)	Sigma-Aldrich	T4174
DMSO	Sigma-Aldrich	D2438
Tetracycline hydrochloride	Sigma Aldrich	T7660

For the preparation of 1X Trypsin/EDTA, the 10X solution is diluted in PBS (without Ca^{2+} and Mg^{2+}), aliquoted and stored in the freezer.

For the preparation of blasticidin, the powder is diluted in dH_2O to achieve a stock concentration of 15 mg/mL, filter sterilized and stored frozen.

For the preparation of tetracycline, the powder is diluted in dH_2O to achieve a stock concentration of 1 mg/mL, filter sterilized, aliquoted and stored in the freezer. (Do not store for more than 6 months!)

2.2 Recommended Complete Medium

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

2.3 Antibiotics

- To cultivate CHO Nav1.5 sodium channel cells, 15 µg/mL blasticidin and 100 µg/mL zeocin should be used.

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

2.4 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 at least 4-6 hours to allow the cells to attach to the bottom of the flask
- Once cells attach to the bottom of the flask and look healthy and proliferate, aspirate off the medium and replace with 5 mL complete medium containing selection antibiotics for cultivation (see 2.3)
- To check whether cells are attached properly, the flask can be gently moved while looking under the microscope
- If 48h after thawing the confluency is below 50%, replace the medium in the flask with fresh medium containing antibiotics
- Incubate cells at 37°C and check them daily until 60% to 80% confluency is reached.

2.5 Splitting Cells

- When cells are 50% to 80% confluent remove complete medium.
- Wash cells with 1xPBS to remove excess medium
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C.
- Detach cells by gently tapping the sides of the flask 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.

2.6 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).

2.7 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% to 90% confluency prior to freezing.
- Remove the complete medium and wash cells with 1xPBS.
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C
- Detach cells by gently tapping the sides of the flask, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells at 200 g using a centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 1.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.75 mL of cell suspension into each cryovial.
- Overnight incubate cells in a polystyrene box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

3 SEQUENCE

Cloned cDNA sequence of human $Na_V1.5$ subunit was error-free and encodes for NP_000326.2:

MANFLLPRGTSSFRRFTRESLAAIEKRMAEKQARGSTTLQESREGLPEEEAPRPQLDLQASKKLPDLYGNPPQELIGEPLEDLDPFYSTQKTFIVLNKGKTI
FRFSATNALYVLSPFHPIRRAAVKILVHSLFNMLIMCTILTNCVFMAQHDPPPWTKYVEYTFTAIYTFESLVKILARGFCLHAFTFLRDPWNWLDFSVIIM
AYTTEFVDLGNVSALRTFRVLRALKTISVISGLKTIVGALIQSVKKLADVMVLTVFCLSVFALIGLQLFMGNLRHKCVRNFTALNGTNGSVEADGLVWESL
DLYLSDPENYLLKNGTSDVLLCGNSSDAGTCPEGYRCLKAGENPDHGYTSFDSFAWAFLALFRLMTQDCWERLYQQTLRSAGKIYMIFFMLVIFLGSFY
LVNLILAVVAMAYEEQNQATIAETEEKEKRFQEAMEMLKKEHEALTIRGVDTVSRSSLEMSPLAPVNSHERRSKRRKRMSSGTEECGEDRLPKSDSED
GPRAMNHLSLTRGLSRTSMKPRSSRGSIFTFRRRDLGSEADFADDENSTAGESESHHTSLLVPWPLRRTSAQGQPSPGTSAPGHALHGKKNSTVDCN
GVVSLLGAGDPEATSPGSHLLRPVMLEHPPDTTTPSEEPGGPQMLTSQAPCVDGFEEPGARQRALSAVSVLTSALEELEESRHKCPPCWNRLAQRYLI
WECCPLWMSIKQGVKLVVMDPFTDLTITMCIVLNTLFMALEHYNMTSEFEEMLQVGNLVFTGIFTAEMTFKIIALDPYYYFQQGWNIFDSIIVILSLMEL
GLSRMSNLSVLRSFRLLRVFKLAKSWPTLNTLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGKNYSELRDSDSGLLPRWHMMDFFHAFLIIFRILCGE
WIETMWDCMEVSGQSLCLLVFLLVMVIGNLVVLNLFLALLLSSFSADNLTAPDEDREMNNLQLALARIQRGLRFVKRTTWDFCCGLLRQRPQKPAALA
AQGQLPSCIATPYSPPPPETEKVPPTRKETRFEEGEQPGQGTPGDPEPVCVPIAVAESDTDDQEEDEENSLGTEEESSKQESQPVSGGPEAPPDSRTWSQ
VSATASSEAEASASQADWRQQWKAEPQAPGCGETPEDSCSEGSTADMTNTAELLEQIPDLGQDVKDPEDCFTEGCVRRCPCCAVDTTQAPGKVWW
RLRKTCYHIVEHSWFETFIIFMILLSSGALAFEDIYLEERKTIKVLLEYADKMFTYVFVLEMLLKWVAYGFKKYFTNAWCWLDFLIVDVSLVSLVANTLGFA
EMGPIKSLRTLRALRPLRALSRFEGMRVVVNALVGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGKFGRCINQTEGDLPLNYTIVNNKSQCESLNLTGELYW
TKVKVNFDNVGAGYLALLQVATFKGWMDIMYAAVDSRGYEEQPQWEYNLYMYIYFVIFIIFGSFFTLNLFIGVIIDNFNQQKKKLGGQDIFMTEEQKKYY
NAMKKLGSKKPQKPIPRPLNKYQGFIFDIVTKQAFDVTIMFLICLNMVTMMVETDDQSPEKINILAKINLLFVAIFTGECIVKLAALRHYYFTNSWNIFDFV
VVILSIVGTVLSDIIQKYFFSPTLFRVIRLARIGRILRLIRGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYSIFGMANFAYVKWEAGIDDMFNFQTFANSM
LCLFQITTSAGWDGLLSPILNTGPPYCDPTLPNSNGSRGDCGSPAVGILFFTTYIIISFLIVVNMYIAIILENFSVATEESTEPLSEDDFDMFYEIWEKFDPEA
TQFIEYSVLSDFADALSEPLRIAKPNQISLINMDLPMVSGDRIHCMDILFAFTKRVLGESGEMDALKIQMEEKFMAANPSKISYEPITTTLRRKHEEVSAM
VIQRAFRRHLLQRSLKHASFLFRQQAGSGLSEEDAPEREGLIAYVMSENFSRPLGPPSSSSISSTSFPPSYDSVTRATSDNLQVRGSDYSHSEDLADFPP
SPDRDRESIV*

4 CONTACT INFORMATION

4.1 Contact Address for Technical Support & Ordering Information

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