B'SYS GmbH

HEK $Na_V1.5$ *big late* Cell Line

Culture Conditions

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1. BACKGROUND

1.1. The cardiac late sodium current I_{NaL} current

Torsades de pointes (TdP) is a potentially fatal type of a ventricular tachycardia associated with delayed repolarization of the cardiac action potential. The major reason for pharmacologically induced TdP is the blockade of the voltage-gated potassium channel ($K_V11.1$ or hERG current, I_{Kr}). Therefore, the main focus of pre-clinical in vitro tests has been set on detection of I_{Kr} blockade to effectively discard drugs with a propensity to induce TdP. However, not all compounds that block I_{Kr} will eventually induce tachyarrhythmia and, therefore, a detected block of I_{Kr} alone is not specifically predictive for delayed repolarization and TdP. Not all known I_{Kr} blockers cause significant arrhythmia because effects caused by induced reduction of potassium outward currents may be counterbalanced by a reduced calcium inward current (I_{CaL}) or late inward sodium current (I_{NaL}).

1.2. B'SYS's HEK $Na_V1.5$ *big late* Cells

Since the physiological late sodium current exhibits only tiny current amplitudes, I_{NaL} needs to be increased for drug screening by decreasing or slowing the inactivation of $Na_V1.5$ channels. For this purpose B'SYS has designed a HEK $Na_V1.5$ cell line containing mutations resulting in the following amino acid exchanges L409C/A410W (Wang & Wang 2005, Wang et al. 2006). These exchanged amino acids were found to lead to a inactivation-deficient $Na_V1.5$ channel. The mutations are localized in D1S6 and presumably prevents access of the intrinsic fast inactivation particle to the inner cavity.

2. PRODUCT SHIPMENT

2.1. Product format

HEK cells stably transfected with recombinant $Na_V1.5$ *big late* channel:

- 1 x 0.5mL aliquots of frozen cells at 2.3 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. CULTURE CONDITIONS

3.1. General

HEK $Na_V1.5$ *big late* 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 1.0 µg/mL Puromycin. The HEK $Na_V1.5$ *big late* cells are passaged at a confluence of about 80%.

- 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% confluence at 1:3 to 1:5 ratio.

3.2. Recommended Complete Medium

- DMEM/F12 with Glutamine or GlutaMax
- 10% FBS
- 1.0% Penicillin/Streptomycin

3.3. Antibiotics

- HEK $Na_V1.5$ *big late* clones were selected under 5.0 µg/mL Puromycin antibiotic pressure.
- To cultivate HEK $Na_V1.5$ *big late* cells, also a reduced antibiotic pressure (1.0 µg/mL Puromycin) can be used.

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

3.4. 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: 1.0 µg/mL Puromycin.
- Incubate cells and check them daily until 70% - 80% confluence is reached.

3.5. 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.

3.6. 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).

Alternatively sterile DMSO can be mixed with complete medium without filtering.

3.7. Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 90% confluence 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 polystyrene box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

4. CONTACT INFORMATION

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