ION BIOSCIENCES

Instructions

CHO-K1 K_V11.1 (hERG) Recombinant cell line

Catalog No. C1202

Introduction

Voltage-gated potassium channels (K_V) are transmembrane channels specific for potassium and sensitive to changes in a cell's membrane potential. All mammalian K_V channels consist of four α-subunits, each containing six transmembrane α helical segments (S1-S6) and a membrane-reentering P-loop, which are arranged circumferentially around a central pore. The human genome contains 40 voltage-gated potassium channels, which are classified into 12 subfamilies, K_V1-K_V12. K_V channels are involved in diverse physiological processes ranging from repolarization of neuronal and cardiac action potentials, over regulating calcium signaling and cell volume, to driving cellular proliferation and migration. Thus, K_V channels offer tremendous opportunities for the development of new drugs for cancer, autoimmune diseases and metabolic, neurological and cardiovascular disorders.

Recombinant CHO-K1 K_V11.1 (hERG) cell line expresses human KCNH2 (voltage-gated potassium channel subfamily H member 2, accession number NM_000238). K_V11.1 channels are abundantly expressed in the heart and brain. In the heart, Kv11.1 channels plays a crucial role in repolarization of the cardiac action potential; inhibition of K_V11.1 current due to channelopathies or inhibitors causes drug-induced long QT syndrome, a potentially fatal cardiac arrhythmia. K_V11.1 can be blocked by a large variety of structurally diverse compounds, thus the FDA requires that all new drug candidates are tested for K_V11.1 inhibition. Besides its relevance in cardiac physiology, K_V11.1 channels are also linked to schizophrenia, tumor cell proliferation, regulation of cell migration and apoptosis. Our CHO-K1 Kv11.1 (hERG) cell line is suitable for discovering modulators of Kv11.1 channel activity and screening for Kv11.1 activators/inhibitors in high-throughput assays.

Materials Provided

One vial of CHO-K1 Kv11.1 (hERG) recombinant cells, 2 x 10^6 cells in 1 mL of Bambanker® Serum-Free Freezing Medium.

Storage

Cells are shipped on dry ice and should arrive frozen. To ensure maximum cell viability, store the cell vial in liquid nitrogen immediately upon receipt. (Liquid nitrogen vapor phase only - vials are not rated for liquid immersion).

Mycoplasma testing

The cell line has been screened using ATCC PCR-based testing service which covers 60 species of *Mycoplasma, Acholeplasma, Spiroplasma* and *Ureaplasma* including the eight species most likely to afflict cell cultures: *M. arginini, M. fermentans, M. hominis, M.hyorhinis, M. orale, M. pirum, M. salivarium,* and *A. laidlawii.* The absence of Mycoplasma species has been confirmed for each lot.

Materials Required But Not Supplied

Category	Name	Recommendations
Reagents	Cell culture media	Gibco™ 1X Advanced MEM (ThermoFisher Cat#: 12492013)
	L-glutamine	Gibco™ 100X GlutaMAX™ Supplement (ThermoFisher Cat#: 35050061)
	Fetal bovine serum (FBS)	Corning™ Premium Fetal Bovine Serum (FisherSci Cat#: MT35016CV)
	Puromycin	Gibco™ Puromycin Dihydrochloride (ThermoFisher Cat#: A1113803)
	Trypsin-EDTA	Gibco™ 1X TrypLE™ Express Enzyme (ThermoFisher Cat#: 12605028)
	Phosphate buffered saline	Gibco™ 1X PBS, pH 7.4 (ThermoFisher Cat#: 10010023)
	Freezing media	Bambanker® Serum-Free Cell Freezing Medium (FisherSci Cat#: NC2960954)
Equipment	Single and multichannel micropipettes and pipette tips	
	50 mL and 15 mL conical centrifuge tubes	
	1.5 mL microtubes	
	Tissue culture disposable pipettes (1 mL-25 mL) and tissue culture flasks (e.g. T75 flask)	
	0.2 µm filter unit(s) for medium sterilization	
	Cryovials, cryo-labels, and −1°C/minute Freezing Container for freezing cells	
	Automated Cell Counter (or Hemocytometer)	
	Humidified tissue culture incubator (37°C and 5% CO_2)	

Cell Culture Protocol

A. Cell culture media

1. Make **Thawing Medium** by combining 25 mL of FBS, 5 mL of 100X GlutaMax™ with 470 mL 1X Advance MEM media for a final concentration of 5% FBS and 1X GlutaMax™.
2. To make **Complete Cell Culture Medium**, add 150 µL of 10 mg/mL Puromycin into 500 mL of Thawing Medium from **step 1** (or 15 µL of Puromycin for every 50 mL of Thawing Medium) for a final concentration of 3 µg/mL Puromycin.
3. (Optional) Sterilize all media using a 0.2 µm filter.
4. Pre-warm all media in a 37°C water bath prior to use.

B. Thawing cells

1. Aliquot 8 mL of **Thawing Medium** in a 15 mL conical centrifuge tube.
2. Place the cryovial in a 37°C water bath briefly, until only small ice crystals remain and the cell pellet is almost completely thawed. The thawing time typically ranges from 2 to 3 minutes. Do **NOT** vortex freshly thawed cells.
3. Spray and wipe the external surface of the cryovial with 70% ethanol. Transfer the vial to a biosafety cabinet.
4. Gently transfer the cells into the pre-filled 15 mL conical tube dropwise.
5. Rinse the cryovial with 1 mL of cell suspension to maximize cell recovery and add it back into the 15 mL conical tube.

Cell Culture Protocol (Continued)

6. Centrifuge the 15 mL conical tube containing cells at 500 x g for 5 min at 25°C.
7. Add 14 mL of **Complete Cell Culture Medium** into a T75 flask.
8. After centrifugation, carefully aspirate the supernatant without disturbing the cell pellet.
9. Gently resuspend the cell pellet with 1 mL of **Complete Cell Culture Medium**.
10. Transfer the cell suspension to the T75 flask and gently swirl the flask to distribute the cells evenly in solution.
11. Incubate the flask in a 37°C and 5% CO2 humidified cell culture incubator.
12. Maintain the cells in culture by exchanging culture media every 2-3 days, until they reach >80% confluency in a T75.

C. Cell passage

1. Remove the T75 flask from the tissue culture incubator and place it in a biosafety cabinet.
2. Gently aspirate the media from the T75 flask.
3. Add 15 mL of PBS into the T75 flask, and gently rock the flask back and forth to rinse the cells.
4. Gently aspirate PBS from the flask.
5. Add 3 mL of pre-warmed TrypLE™ to the flask. Gently rock the flask back and forth to ensure that the flask is uniformly covered with TrypLE™.
6. Incubate the flask at 37°C and 5% CO_2 until the cells have detached. Usually it will take 8 - 10 min.
7. Remove the flask from the incubator and observe the cells under a microscope to confirm cell detachment. If necessary, gently tap the edge of the flask to detach cells from the surface.
8. Add 10 mL of pre-warmed **Complete Cell Culture Medium** to the detached cells in the flask.
9. Transfer cell suspension into a 15 mL conical tube.
10. Centrifuge the 15 mL conical tube containing cells at 500 x g for 5 min at 25°C.
11. After centrifugation, carefully aspirate the supernatant without disturbing the cell pellet.
12. Resuspend the cell pellet with 5 mL of **Complete Cell Culture Medium**. Pipette the cell suspension up and down several times to generate a single cell suspension without any clumps.
13. Take a small aliquot of the cell suspension (~20 µL) for cell counting, and determine the number of cells and volume required to seed into a T75 flask. CHO-K1 K_V11.1 (hERG) cell doubling time is ~24h. A confluent T75 flask normally yields ~10 x 10^6 cells. We recommend to seed 1 x 10^6 cells for a T75 flask.
14. Add 15 mL of **Complete Cell Culture Medium** into a new T75 flask, followed by addition of the appropriate volume of cell suspension. Transfer the flask to a tissue culture incubator, and incubate the cells at 37°C and 5% CO_2.

D. Cryopreservation

1. Harvest cells according to the description in **section C**, Cell Passage **step 1 - 9**.
2. Set aside a small fraction of the suspended cells (100 µL or less) in a separate tube for cell counting.
3. Count cells, calculate the concentration of cells and the total number of cells in the original 15 mL conical tube.

Cell Culture Protocol (Continued)

4. Centrifuge the 15 mL conical tube containing cells at 500 x g for 5 min at 25°C.
5. After centrifugation, gently aspirate and discard the supernatant, being careful not to disturb the cell pellet.
6. Based on the total cell number calculated in **step 3**, resuspend the cells to the desired concentration (e.g. 2.0 x 10^6 cells/mL) in Bambanker® Serum-Free Cell Freezing Medium.
7. Aliquot 1 mL of the cell suspension into labeled 2 mL cryovials. Seal the cryovials tightly.
8. Freeze cells in a -80°C freezer at a controlled rate of -1°C/minute overnight in a commercially-available freezing container.
9. The following day, transfer the vials into the vapor phase of liquid nitrogen for long-term storage.

Functional Validation

The biological function of K_V11.1 channels were validated with a known channel blocker, terfenadine, using ION's Brilliant Thallium Flux assay. Terfenadine is a highly selective blocker that interact with K_V11.1 residues inside the pore. The reported IC50 of terfenadine is 204 nM. CHO-K1 K_V11.1 (hERG) cells are first incubated with Thallos AM dye solution for 1 hour. Then cells are treated with various concentrations of Terfenadine for 15 min. For the assay readout, high concentration potassium buffer is added to the stimulus solution to depolarize cells and activate K_V11.1 channels. While the channels open, extracellular Tl^+ ions flow into the cells and bind to Thallos, increasing fluorescence. Baseline fluorescence is first collected for 20 sec, then fluorescence continues to be acquired for 280 sec after the high potassium thallium stimulus solution addition (Figure 1). V_{max} is calculated as the slope of the first 10 time points right after the stimulus solution addition. Dose response curves were plotted as Log terfenadine concentration against V_{max}; generating an IC_{50}: 176 nM which is consistent to the reported value.

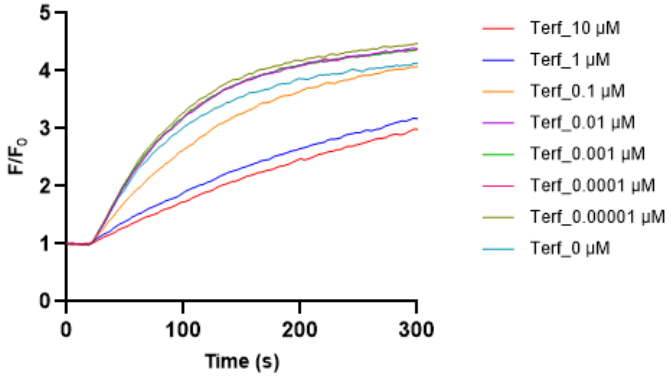

Figure 1. Terfenadine Dose Response Curve kinetic data. Thallium flux assay was performed using FlexStation 3 kinetic plate reader with excitation wavelength 490 nm and emission wavelength 520 nm. Baseline RFUs were collected for 20s, then thallium stimulus was added to the cell plate. Fluorescence was baseline normalized (F/F_0). V_{max} was calculated as the slope of the first 10 data points after the high potassium thallium stimulus solution addition.

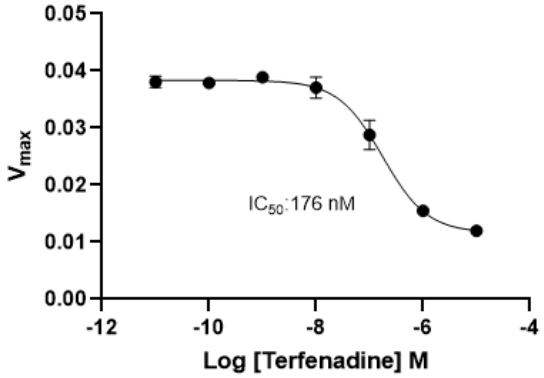

Figure 2. Terfenadine Dose Response Curve. Thallium flux assay was performed using FlexStation 3 kinetic plate reader with excitation wavelength 490 nm and emission wavelength 520 nm. CHO-K1 Kv11.1 (hERG) cells were treated with potassium channel inhibitor Terfenadine at the highest concentration of 10 µM. Dose response curves were plotted as Log terfenadine concentration against V_{max}; generating an IC_{50}: 176 nM.

Vector and Sequence

Vector description

Mammalian Gene Expression PiggyBac Vector, pRP[Exp]-Puro-CAG>hKCNH2[NM_000238.4]

hKCNH2 sequence (NM_000238.4)

MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVMQRPCTCDFLHGPRTQRRAAAQIAQALLGAE
ERKVEIAFYRKDGSCFLCLVDVVPVKNEDGAVIMFILNFEVVMEKDMVGSPAHDTNHRGPPTSWLAPGRAKTFRLKLPALLALTARESS
VRSGGAGGAGAPGAVVVDVDLTPAAPSSESLALDEVTAMDNHVAGLGPAEERRALVGPGSPPRSAPGQLPSPRAHSLNPDASGSSCS
LARTRSRESCASVRRASSADDIEAMRAGVLPPPPRHASTGAMHPLRSGLLNSTSDSDLVRYRTISKIPQITLNFVDLKGDPFLASPTSDRE
IIAPKIKERTHNVTEKVTQVLSLGADVLPEYKLQAPRIHRWTILHYSPFKAVWDWLILLLVIYTAVFTPYSAAFLLKETEEGPPATECGYAC
QPLAVVDLIVDIMFIVDILINFRTTYVNANEEVVSHPGRIAVHYFKGWFLIDMVAAIPFDLLIFGSGSEELIGLLKTARLLRLVRVARKLDRYS
EYGAAVLFLLMCTFALIAHWLACIWYAIGNMEQPHMDSRIGWLHNLGDQIGKPYNSSGLGGPSIKDKYVTALYFTFSSLTSVGFGNVSPN
TNSEKIFSICVMLIGSLMYASIFGNVSAIIQRLYSGTARYHTQMLRVREFIRFHQIPNPLRQRLEEYFQHAWSYTNGIDMNAVLKGFPECLQ
ADICLHLNRSLLQHCKPFRGATKGCLRALAMKFKTTHAPPGDTLVHAGDLLTALYFISRGSIEILRGDVVVAILGKNDIFGEPLNLYARPGK
SNGDVRALTYCDLHKIHRDDLLEVLDMYPEFSDHFWSSLEITFNLRDTNMIPGSPGSTELEGGFSRQRKRKLSFRRRTDKDTEQPGEVSA
LGPGRAGAGPSSRGRPGGPWGESPSSGPSSPESSEDEGPGRSSSPLRLVPFSSPRPPGEPPGGEPLMEDCEKSSDTCNPLSGAFSGVS
NIFSFWGDSRGRQYQELPRCPAPTPSLLNIPLSSPGRRPRGDVESRLDALQRQLNRLETRLSADMATVLQLLQRQMTLVPPAYSAVTTP
GPGPTSTSPLLPVSPLPTLTLDSLSQVSQFMACEELPPGAPELPQEGPTRRLSLPGQLGALTSQPLHRHGSDPGS

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