ION BIOSCIENCES

Instructions

HEK293 K_V11.1 (hERG) Cell Line

Catalog No. C1103

Introduction

Voltage-gated potassium (K_V) channels are integral membrane proteins that enable selective potassium ion permeation in response to changes in cellular membrane potential. These channels play critical roles in shaping action potentials, regulating cellular excitability, and maintaining ion homeostasis across diverse tissues. The human genome encodes 40 K_V channels, categorized into 12 subfamilies (K_V1–K_V12), each exhibiting unique biophysical properties and tissue distribution.

K_V11.1, also known as hERG (human Ether-à-go-go Related Gene), is a key member of the K_V channel family encoded by the KCNH2 gene. This channel is predominantly expressed in cardiac myocytes and the central nervous system, where it is essential for repolarizing the cardiac action potential and regulating neuronal excitability. Dysfunction or pharmacological inhibition of K_V11.1 channels leads to delayed cardiac repolarization, manifesting as drug-induced long QT syndrome—a potentially fatal arrhythmia of major concern in drug development. Because of this, regulatory agencies, including the FDA, mandate routine screening of new drug candidates for K_V11.1 inhibition. Beyond cardiac physiology, K_V11.1 channels have emerging roles in neurological disorders, tumor proliferation, and cellular migration, making them a valuable target for a broad range of therapeutic applications.

ION Biosciences' HEK293 K_V11.1 (hERG) recombinant cell line stably expresses human KCNH2 and provides a robust, well-validated model for investigating K_V11.1 channel function. This cell line is ideally suited for high-throughput screening of channel modulators, safety pharmacology assays, and mechanistic studies aimed at discovering novel inhibitors or activators of K_V11.1. With excellent reproducibility and functional expression, these cells facilitate efficient evaluation of compound effects on K_V11.1 currents using fluorescence-based assays.

Materials Provided

One vial of HEK293 K_V11.1 (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[1]	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)
	Dissociation Reagent	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)	

[1] *If desired, antibiotics such as Penicillin and Streptomycin may be supplemented to prevent bacterial infection. We recommend using Gibco™ Penicillin Streptomycin GlutaMAX™ Supplement (Thermo Fisher Cat#: A5873601) in place of GlutaMAX™.*

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 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.

Cell Culture Protocol (Continued)

4. Gently add the cells into the pre-filled 15 mL conical tube dropwise.
5. Rinse the cryovial with 1 mL of **Thawing Medium** to maximize cell recovery and add it back into the 15 mL conical tube.
6. Centrifuge the 15 mL conical tube containing the cells at 500 x g for 5 min at 25°C.
7. After centrifugation, carefully aspirate the supernatant without disturbing the cell pellet.
8. Gently resuspend the cell pellet with 1 mL of **Complete Cell Culture Medium**.
9. Add 14 mL of **Complete Cell Culture Medium** into a T75 flask.
10. Transfer the cell suspension to the T75 flask and gently swirl the flask to distribute the cells evenly in the solution.
11. Incubate the flask in a 37°C and 5% CO_2 humidified cell culture incubator.
12. Maintain the cells in culture by changing culture medium 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. HEK293 K_V11.1 (hERG) cells have a doubling time of ~24 hours. A confluent T75 flask normally yields ~10 x 10^6 cells. We recommend to seed 1 x 10^6 cells for a T75 flask in three to four days.
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.

Cell Culture Protocol (Continued)

D. Cryopreservation

1. Harvest cells according to the description in **Section C Cell Passage steps 1 - 9**.
2. Set aside a small fraction of the suspended cells (~20 µL) 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.
4. Centrifuge the 15 mL conical tube containing cells at 500 x g for 5 min at 25°C.
5. After centrifugation, 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 cell 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 the HEK293 K_V11.1 (hERG) recombinant cell line was validated using terfenadine, a well-characterized tool compound known to inhibit hERG function. Functional activity of hERG channels was measured using ION Biosciences' Brilliant Thallium Gold Assay, Brilliant Thallium Gold Snapshot Assay, and Thallium-Free hERG Potassium Channel Assay kits. These kits use fluorescent ion indicators to detect the flow of thallium or potassium ions through the channel. HEK293 K_V11.1 (hERG) recombinant cells were pre-incubated with the fluorescent ion indicators' optimized loading conditions, followed by exposure to increasing concentrations of terfenadine. A dose-dependent change in fluorescence was observed upon addition of a membrane-depolarizing stimulus solution, reflecting inhibition of K_V11.1-mediated ion flux. Fluorescence was recorded continuously following compound addition, and the resulting data were analyzed to determine IC_{50} values based on maximum velocity (V_{max}) or area under the curve (AUC). The assay produced an IC_{50} consistent with literature values for terfenadine, confirming functional expression and pharmacological responsiveness of K_V11.1 channels in the HEK293 cell line.

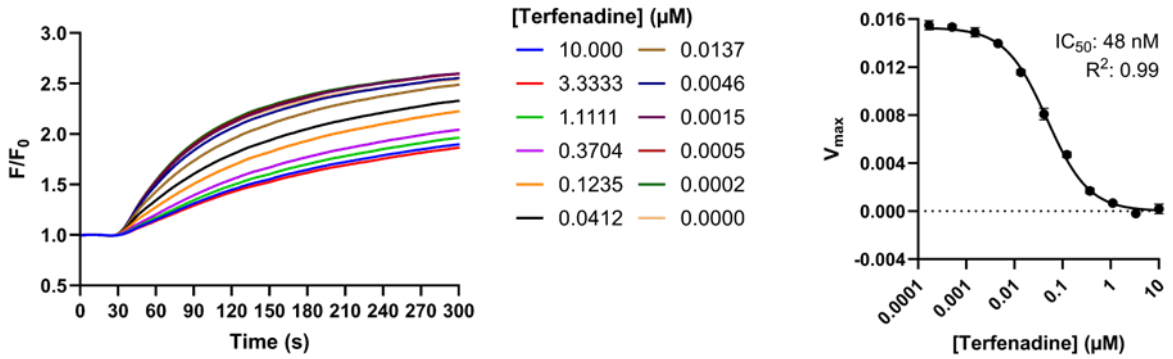

Figure 1. Terfenadine dose response on HEK293 K_V11.1 (hERG) recombinant cell line using ION Biosciences' Brilliant Thallium Gold Assay[2]. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Terfenadine, a potent hERG channel inhibitor, was added 15 minutes before the start of the assay. At 30 seconds, a membrane-depolarizing stimulus solution was added. The V_{max} was calculated from the first 10 seconds following stimulus addition. Error bars indicate the SEM (n = 3). The IC_{50} for terfenadine inhibition of K_V11.1 is about 48 nM.

Functional Validation (Continued)

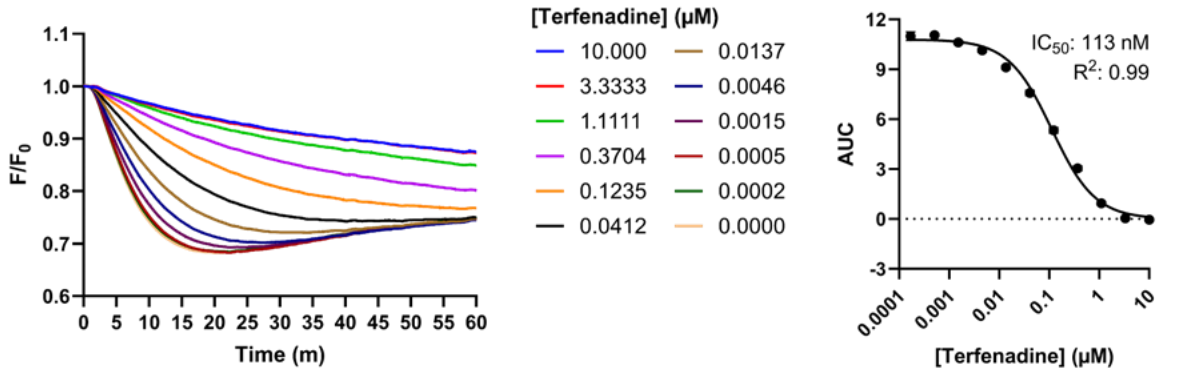

Figure 2. Terfenadine dose response on HEK293 K_V11.1 (hERG) recombinant cell line using ION Biosciences' Brilliant Thallium Gold Snapshot Assay[2]. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Terfenadine, a potent hERG channel inhibitor, was added 15 minutes before the start of the assay. At 30 seconds, a membrane-depolarizing stimulus solution was added. The AUC was calculated from the baseline at y=1 to the curve. Error bars indicate the SEM (n = 3). The IC_{50} for terfenadine inhibition of K_V11.1 is about 113 nM.

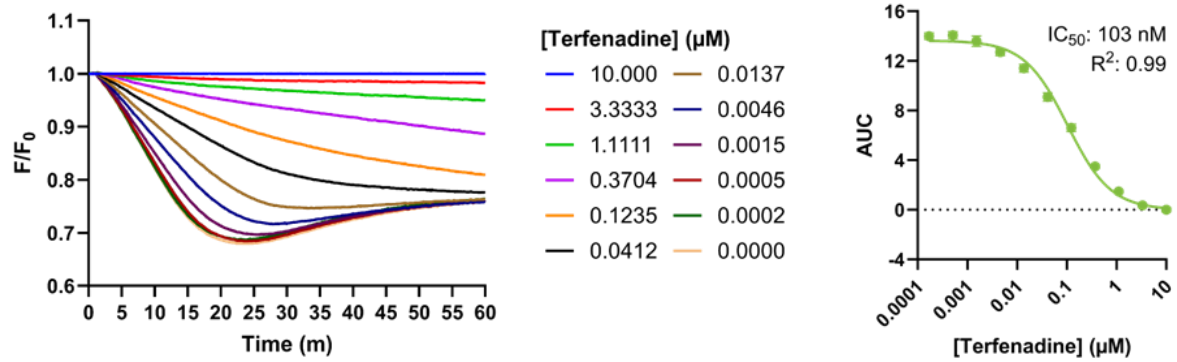

Figure 3. Terfenadine dose response on HEK293 K_V11.1 (hERG) recombinant cell line using ION Biosciences' Thallium-Free hERG Potassium Channel Assay. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Terfenadine, a potent hERG channel inhibitor, was added 15 minutes before the start of the assay. At 30 seconds, a membrane-depolarizing stimulus solution was added. The AUC was calculated from the baseline at y=1 to the curve. Error bars indicate the SEM (n = 3). The IC_{50} for terfenadine inhibition of K_V11.1 is about 103 nM.

[2] Compatible Assay Kits

ION Biosciences has Assay Kits available that provide all the reagents needed for testing your K_V11.1 channel modulators for both 96 and 384 well plate formats, and are compatible with our HEK293 K_V11.1 (hERG) cell line. Assay kits are available for direct purchase from our website or by contacting our Sales Department.

Name	Sizes	Catalog #
Brilliant Thallium Assay Kit	pIONeer - 2 Plates Flex - 10 Plates Express - 100 Plates	11000-2 11000-10 11000-100
Brilliant Thallium Gold Assay Kit	pIONeer - 2 Plates Flex - 10 Plates	11020-2 11020-10
Brilliant Thallium Snapshot Assay Kit	pIONeer - 2 Plates Flex - 10 Plates	11011-2 11011-10
Brilliant Thallium Gold Snapshot Assay Kit	pIONeer - 2 Plates Flex - 10 Plates	11021-2 11021-10
Flow Cytometric Potassium Channel Assay kit	200 Assays	11015-1
Thallium-free hERG Potassium Channel Assay Kit	pIONeer - 2 Plates Flex - 10 Plates	12100-2 12100-10

Vector and Sequence

Vector description

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

hKCNH2 sequence [NM_000238.4]

MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVMQRPCTCDFLHGPRTQRRAAAQIAQALLGA
EERKVEIAFYRKDGSCFLCLVDVVPVKNEDGAVIMFILNFEVVMEKDMVGSPAHDTNHRGPPTSWLAPGRAKTFRLKLPALLALTARES
SVRSGGAGGAGAPGAVVVDVDLTPAAPSSESLALDEVTAMDNHVAGLGPAEERRALVGPGSPPRSAPGQLPSPRAHSLNPDASGSS
CSLARTRSRESCASVRRASSADDIEAMRAGVLPPPPRHASTGAMHPLRSGLLNSTSDSDLVRYRTISKIPQITLNFVDLKGDPFLASPTS
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SGAFSGVSNIFSFWGDSRGRQYQELPRCPAPTPSLLNIPLSSPGRRPRGDVESRLDALQRQLNRLETRLSADMATVLQLLQRQMTLVPP
AYSAVTTPGPGPTSTSPLLPVSPLPTLTLDSLSQVSQFMACEELPPGAPELPQEGPTRRLSLPGQLGALTSQPLHRHGSDPGS

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