Description

Recombinant HEK293 cell line expressing human ERG potassium channel (ether-a-go-go-related gene, also known as KCNH2 or Kv11.1, accession number NM_000238).

Background

hERG (human ether-a-go-go-related gene) encodes the alpha subunit of a potassium ion channel, Kv11.1. It contains six transmembrane α-helices with a re-entrant "pore-loop" between the fifth and the sixth transmembrane helices. This ion channel is best known for its contribution to the electrical activity of the heart that coordinates the heart's beating. When this channel's ability to conduct electrical current across the cell membrane is inhibited or compromised, either by application of drugs or by rare mutations, it can result in a potentially fatal disorder called long QT syndrome. A number of clinically successful drugs in the market exhibit the potential to inhibit hERG, and create a concomitant risk of sudden death as a side-effect, which has made hERG inhibition an important off-target that must be avoided during drug development.

Materials Provided

Components	Format
2 vials of frozen cells	~1.5 X 10^6 cells in 1 ml of 10% DMSO

Host Cell

HEK293

Mycoplasma Testing

The cell line has been screened using the PCR-based VenorGeM® Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2. BPS cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

Growth Medium 1G (BPS Bioscience #79531):

Thaw Medium 1 plus 400 µg/ml of Geneticin (Life Technologies #11811031) to ensure that recombinant expression is maintained. hERG-HEK293 cells should exhibit a typical cell division time of ~24 hours.

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to swirl the frozen cells for 30-40 seconds in a 37°C water-bath, then use 1-2 ml Thaw Medium 1 to completely thaw the cells. Transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin**). Transfer to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin**)
2. Spin down cells, and resuspend cells in pre-warmed Thaw Medium 1 (no geneticin). Transfer resuspended cells to a T25 flask and culture in a CO2 incubator at 37°C overnight.
3. After 24 hours of culture, replace the medium with fresh Thaw Medium 1 (**no Geneticin**), and continue growing culture in the CO2 incubator at 37°C until the cells are ready to be split. Cells should reach ~80% confluence roughly two days after being thawed.
4. Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 1G (**contains Geneticin**).

Cell Passage

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1G (**contains Geneticin**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1G and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:10 to 1:20 weekly or twice per week.

Validation Data

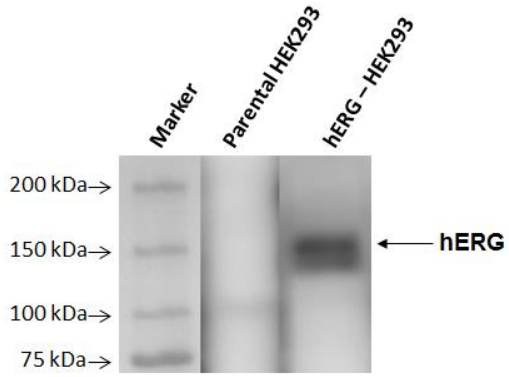
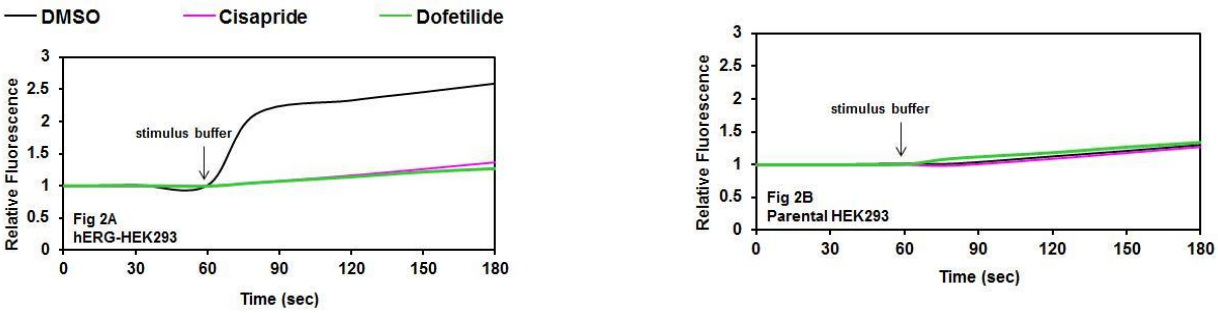


Figure 1. Western blot of hERG. hERG-HEK293 cells were probed with anti-hERG antibody (ThermoFisher # PA3860). Human ERG channel has been stably expressed in HEK293 cell line and its expression was confirmed by Western blotting.

Channel Activity Assays

The channel activity of hERG was characterized by a fluorescence-based assay using thallium influx as a surrogate indicator of potassium ion channel activity coupled with a thallium-sensitive fluorescent dye. When hERG-HEK293 cells were pre-loaded with thallium-sensitive dye and stimulated with stimulus buffer containing potassium/thallium, thallium ions flowed through the open hERG channels into the cells and bound the dye, generating a fluorescent signal. The hERG channel activity in hERG-HEK293 cells was blocked by hERG channel blockers, cisapride or dofetilide, causing the fluorescent signal triggered by thallium influx to drop to the basal level.



A) hERG-HEK293 B) Parental HEK293

Figure 2. Thallium influx in hERG-HEK293 cells is blocked by cisapride or dofetilide. (A) hERG-HEK293 or (B) parental HEK293 cells were loaded with the thallium-sensitive fluorescent dye Thallos (TEFLABS) and treated with DMSO (black), 1 µM of cisapride (pink), or 1 µM of dofetilide (green). Cells were then stimulated (60s) with stimulus buffer containing thallium and potassium. The thallium influx, as a surrogate indicator of hERG channel activity, was measured by Thallos fluoresecence (excitation 490 nm and emission 515 nm).

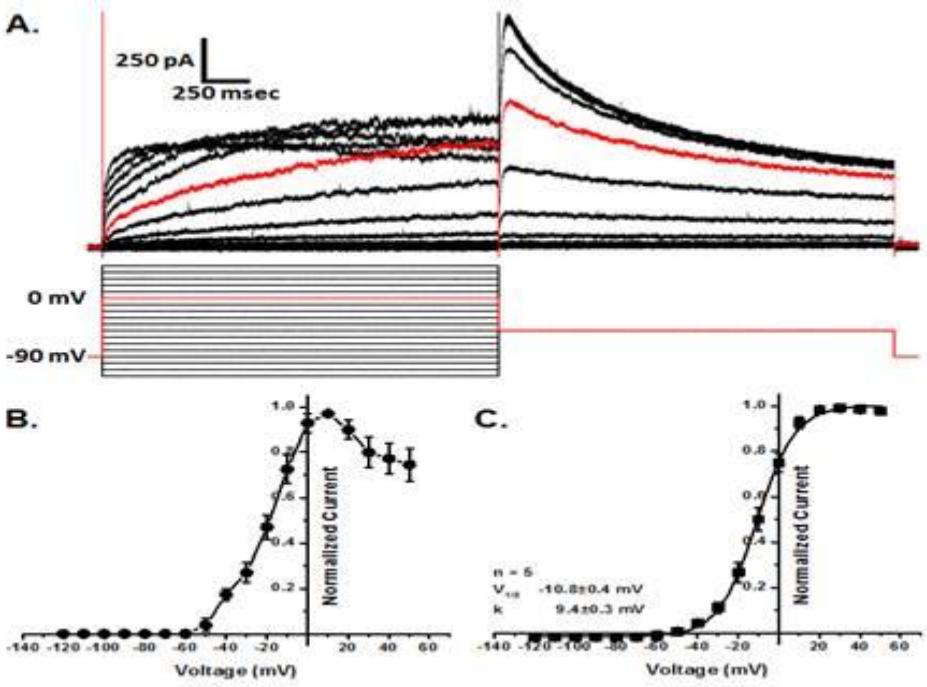


Figure 3. hERG activation traces using hERG (Kv11.1) - HEK293 cells (BPS Bioscience, #60619) in a whole-cell voltage-clamp configuration. Cells were clamped at - 90 mV and stepped to -50 mV with currents recorded over a 2000 ms voltage step from the holding potential to potentials between -120 and 50 mV (P1 pulse) followed by a single voltage step to -50 mV for 2000 ms (P2 pulse). The red trace was obtained at 0 mV. Panels B and C show the P1 activation and P2 steady-state activation current-voltage relationships, respectively. The steady-state activation current-voltage relationships were fit to a Boltzmann function to obtain a V1/2 of -10.8±0.4 mV and a k value of 9.4±0.3 mV (n=5 cells). Data kindly supplied by Dr. Mark Nowak, Cytocybernetics, Pendleton, NY.

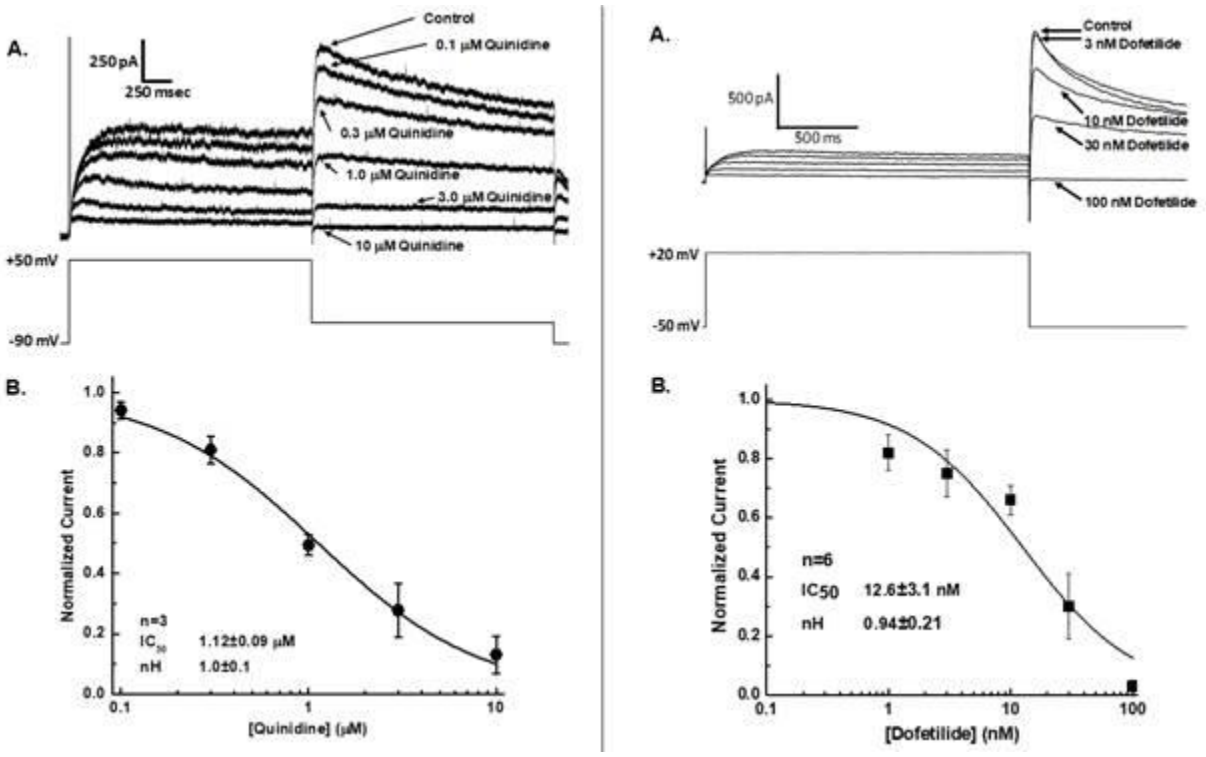


Figure 4. hERG activation traces using hERG (Kv11.1) - HEK293 cells (BPS Bioscience, #60619) in a whole-cell voltage-clamp configuration. Cells were clamped at - 90 mV and stepped to -50 mV with currents recorded over a 2000 ms voltage step from the holding potential to potentials between -120 and 50 mV (P1 pulse) followed by a single voltage step to -50 mV for 2000 ms (P2 pulse). The red trace was obtained at 0 mV. Panels B and C show the P1 activation and P2 steady-state activation current-voltage relationships, respectively. The steady-state activation current-voltage relationships were fit to a Boltzmann function to obtain a V1/2 of -10.8±0.4 mV and a k value of 9.4±0.3 mV (n=5 cells). Data kindly supplied by Dr. Mark Nowak, Cytocybernetics, Pendleton, NY.

Vector and Sequence
Human hERG was cloned into pIRES-neo vector (Clontech).
Polylinker: CMV-EcoRV-NheI-hERG-BamHI-NotI-BstXI-IRES-neomycinR

hERG sequence (accession number NM_000238)

MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVMQRPCTCDFLHGPRTQRRAAAQIAQA
LLGAEERKVEIAFYRKDGSCFLCLVDVVPVKNEDGAVIMFILNFEVVMEKDMVGSPAHDTNHRGPPTSWLAPGRAKTFRLKLPAL
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PGAPELPQEGPTRRLSLPGQLGALTSQPLHRHGSDPGS

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