6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

hERG (Kv11.1) - HEK293 Recombinant Cell line
Cat #: 60619

Product 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).

Format
Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store vials in liquid nitrogen.

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.

Introduction
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.

Culture Conditions
Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) 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.

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Cells should be grown at 37°C with 5% CO_2 using **Growth Medium 1B** (Thaw Medium 1 BPS Cat. #60187) plus 400 μg/ml of Geneticin (Life Technologies #11811031)**.**

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no geneticin)** 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 CO_2 incubator at 37°C overnight. The next day, replace the medium with fresh Thaw Medium 1 **(no geneticin)**, and continue growing culture in the CO_2 incubator at 37°C until the cells are ready to be split. Cells should reach ~80% confluence roughly two days after being thawed. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 1B **(contains geneticin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, and quench trypsin with Growth Medium 1B. Transfer to a tube, spin down cells, aspirate medium, resuspend cells in fresh Growth Medium 1B and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly or twice a week.

Functional validation
Human ERG channel has been stably expressed in HEK293 cell line and its expression was confirmed by Western blotting (Figure 1).

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.

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Figure 1. Western blot of hERG. hERG-HEK293 cells were probed with anti-hERG antibody (ThermoFisher # PA3860).

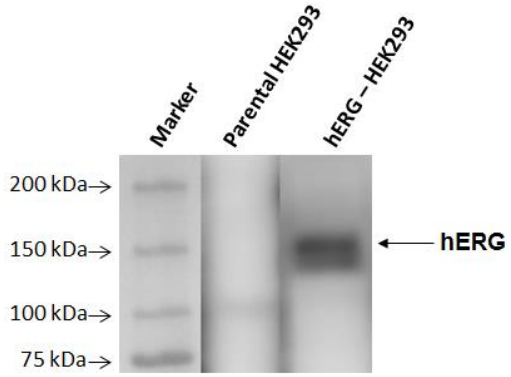
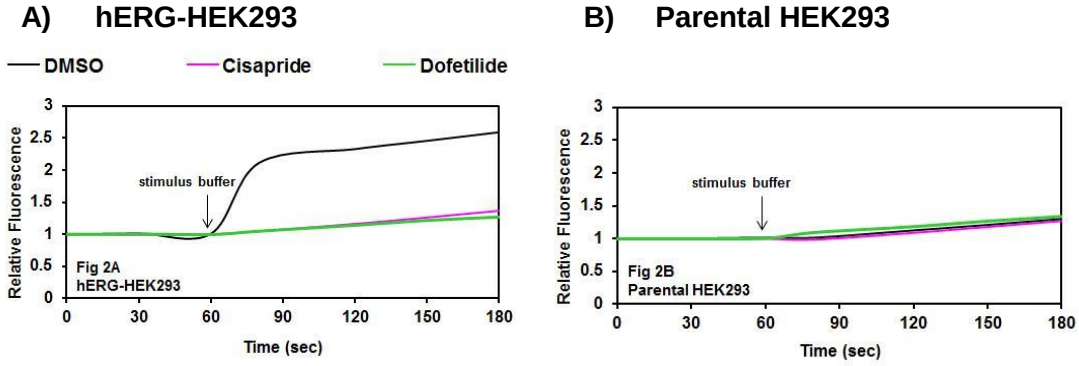


Figure 2. Thallium influx in hERG-HEK293 cells is blocked by cisapride or dofetilide. (A) hERG-HEK293 or **(B)** parental HEK293 cells were loaded with the thallium-sensitive fluorescent dye Thallos (TEFLABS) and treated with DMSO (black), 1 µM of cisapride (pink), or 1 µM of dofetilide (green). Cells 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).



Vector and sequence
Human hERG was cloned into pIRES-neo vector (Clontech).
Polylinker: CMV-EcoRV-NheI-**hERG**-BamHI-NotI-BstXI-IRES-neomycin^{R}

hERG sequence (accession number NM_000238)

MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVMQRPCT
CDFLHGPRTQRRAAAQIAQALLGAEERKVEIAFYRKDGSCFLCLVDVVPVKNEDGAVIMFILNFE
VVMEKDMVGSPAHDTNHRGPPTSWLAPGRAKTFRLKLPALLALTARESSVRSGGAGGAGAPGAVV

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VDVDLTPAAPSSESLALDEVTAMDNHVAGLGPAEERRALVGPGSPPRSAPGQLPSPRAHSLNPDA
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DSLSQVSQFMACEELPPGAPELPQEGPTRRLSLPGQLGALTSQPLHRHGSDPGS

References

Beacham, D.W. *et al*. (2010) Cell-based potassium ion channel screening using the FluxOR assay. *J. Biomol. Screen.* **15(4):**441-446

Murphy, S.M. *et al.* (2006) Evaluation of functional and binding assays in cells expressing either recombinant or endogenous hERG channel. *J. Pharmacol. Toxicol. Methods.* **54(1):**42-55

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