

CreaCell™ hERG HEK293 Cell Line

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A- PRODUCT DESCRIPTION

Recombinant HEK293 cell line expressing the human ERG (ether-a-go-go related gene) potassium channel:

- **Expressed recombinant protein:** Homo sapiens potassium voltage-gated channel, subfamily H (eag-related), member 2 (KCNH2), transcript variant 1, mRNA. cDNA strictly similar to GenBank accession number: NM_000238.
- **Type of expression system:** constitutive (pSG5-Kana).
- **Host cell line:** HEK293.
- **Selection marker:** G418 1.2 mg/ml.
- **Biosafety level:** 2.
- **Mycoplasma testing:** negative.

B- FORMAT AND SHIPPING

- 2 cryogenic vials of 5x10⁶ cells /vial in 90% FBS, 10% DMSO.
- shipping condition: dry ice.

C- CELL CULTURE

C1• Reception of cryovials

Upon reception store cryovials in a liquid nitrogen storage container.

C2• Composition of complete medium

DMEM 4.5 g/L glucose (eg. Invitrogen #21969-035 or PAA #E15-011)

10% Foetal Bovine Serum (FBS) (eg. PAA # A15-151 or #A15-351)

2% glutamine 100 mM (eg. Invitrogen #25030024)

1% penicillin 10.000 U/ml streptomycin 10.000 µg/ml (eg. Invitrogen #15140122)

1.2 mg/ml G418 (eg. Invitrogen #10131-027)

C3 • Protocol - Thawing cells

1. Remove cryogenic vial from liquid nitrogen container and immediately place it into a 37°C water bath until medium is thawed.
2. Disinfect cryogenic vial with 70% ethanol before opening.
3. Transfer thawed cell suspension into a sterile centrifuge tube and add 9 ml of warm complete medium. Centrifuge 5 min at 400 g.
4. Discard supernatant and resuspend cell pellet in 15 ml of complete medium and transfer in T75 flask (minimal concentration: $2 \cdot 10^5$ cells/ml). Grow cells in a humidified incubator at 37°C under 5% carbon dioxide.
5. To maintain electrophysiological performances, cell density must not exceed 80%. Observe at least 48h of delay after thawing of the cells, before analysis with your devices.
6. All 3-4 days, dilute cells.

C4 • Protocol - Passaging cells for culture or patch-clamp (manual or automated)

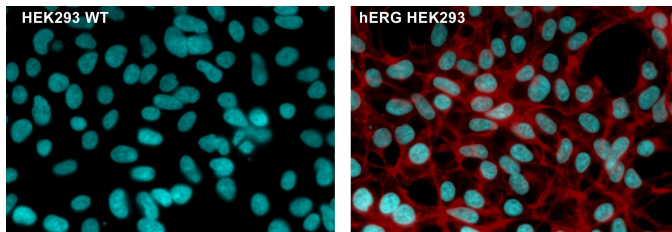
1. Remove poor medium and rinse the adherent cells once with PBS1X.
2. Add 2-3 ml of Accutase™ solution (PAA #L11-007). Place plate on a 37°C warming incubator 3 to 5 min. As soon as cells are detached, add 9 ml of 37°C complete medium. Draw cell suspension into a sterile pipet and homogenize cells gently to dissociate cells aggregates.
3. Count cells using a hemacytometer with Blue Trypan. Centrifuge an appropriate volume for 5 min at 400 g.
4. For culture or manual patch-clamp: discard supernatant and resuspend cell pellet with complete medium before seeding. For automated patch-clamp: discard supernatant and resuspend cell pellet with extra cellular solution (wait for 25 min RT before analysis).

C5 • Protocol - Freezing cells

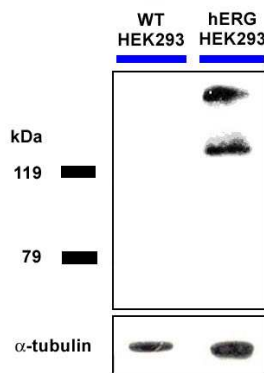
1. Treat cells with Accutase™ (see C4, steps 1 to 3).
2. Remove supernatant and add 1 ml of freezing medium (FBS 90%, DMSO 10%). Resuspend pellet. Dilute with freezing medium as necessary to get a final cells concentration of $5 \cdot 10^6$ cells/ml.
3. Transfer 1-ml aliquots of cell suspension into labeled 2-ml cryogenic vials.
4. Place vials overnight in a cryobox at -80°C, then transfer to liquid nitrogen storage container.

D- BIOCHEMICAL VALIDATION

D1 • Immunofluorescence of wild type HEK293 cells or hERG HEK293 cells with anti-hERG antibody (red). Nucleus staining is performed with Hoechst (blue).



D2 • Western blot analysis of wild type or recombinant HEK293 total cell extracts with anti-hERG antibody.



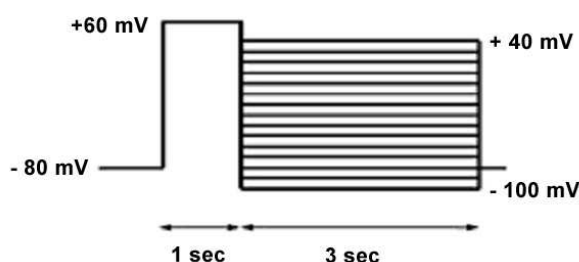
E- PATCH CLAMP VALIDATION

E1 • Solutions:

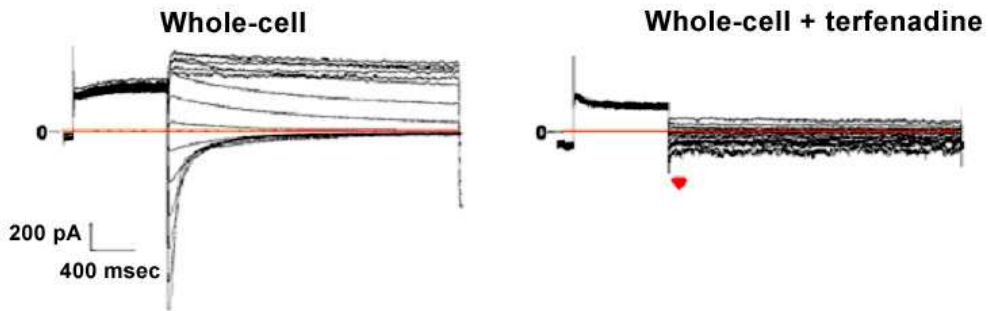
- Extracellular solution: 118 mM NaCl, 5.6 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgCl₂, 10 mM HEPES, 11 mM glucose, pH 7.2
- Intracellular pipet solution: 30 mM KCl, 110 mM K-aspartate, 0.1 mM CaCl₂, 1 mM MgCl₂, 1 mM EGTA, 10 mM HEPES, pH=7.2
- *Terfenadine*: SIGMA T9652.

E2 • Configuration: whole-cell.

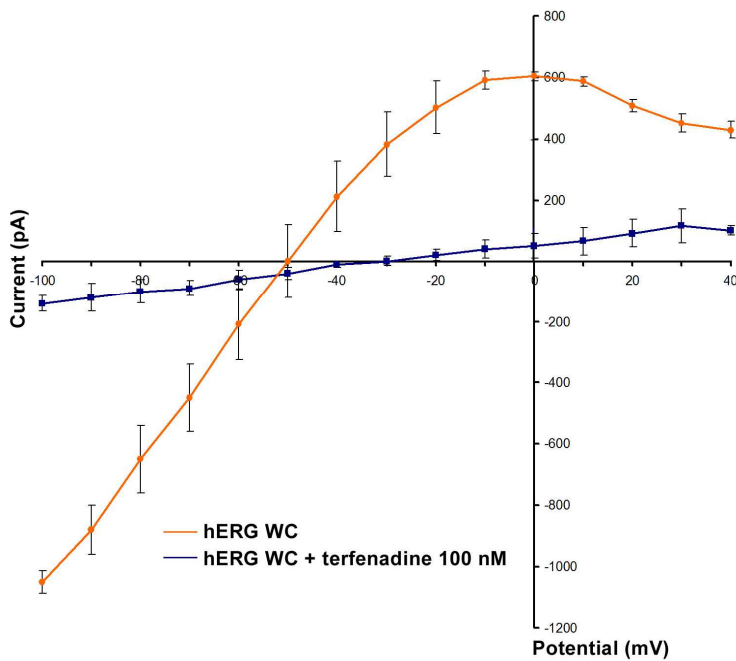
E3 • Recording procedure:



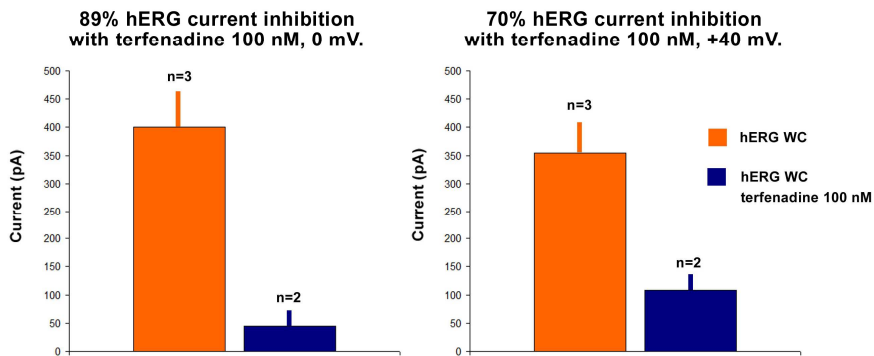
E4 • Currents recorded from hERG HEK293 cell line:



E5 • I-V curves - Terfenadine (100 nM):



E6 • Maximal current inhibition of hERG with 100 nM terfenadine:



E7 • Current stability over continuous culture:

The hERG HEK293 recombinant cell line was propagated over 5 months under the recommended growth conditions. There was no significant variation in expression of the hERG current over this period.

F- LICENSE AGREEMENT

(Extract)

■ The CreaCell™ hERG HEK293 cell line purchased under the Agreement, and/or any Derived Cell Line can be used for research and development and to sell services relating to or using the cell line. For clarity, the Purchaser may use the cell line for commercial purposes in the provision of drug discovery services for itself, its Affiliates, or with third parties (...). The resale of the CreaCell™ hERG HEK293 cell line and/or any Derived Cell Line, in any form, is strictly prohibited.

■ Purchaser shall have a non-transferable right to use the CreaCell™ hERG HEK293 cell line and/or any Derived Cell Line for any purpose, including a right to use such cell line, or fragments thereof, to produce proteins encoded by the cell line. The Agreement does not transfer ownership or title to the CreaCell™ hERG HEK293 cell line, or to any part thereof, to Purchaser.

■ CREACELL warrants that it has the right to supply the CreaCell™ hERG HEK293 cell line and to provide the License to the Purchaser, and that the Purchaser and its Affiliate's use of the CreaCell™ hERG HEK293 cell line under the terms of this Agreement does not infringe any rights including any intellectual property rights belonging to any third party.

■ CREACELL has no claim of any kind to any research, data, facts, information, experimental results, and/or any other embodiments generated by the Purchaser using the CreaCell™ hERG HEK293 cell line or a Derived Cell Line and acknowledges that the Purchaser shall be the exclusive owner of such research, data, facts, information, experimental results, and/or any other embodiments.

■ Should, during the term of the License, Purchaser's stock on CreaCell™ hERG HEK293 cell line disappear for whatsoever reason, CREACELL agrees, as part of the price agreed upon under the Agreement, to provide Purchaser with additional/new stocks of such cell line (shipping and handling charges not included).