

CreaCell™ hERG CHO Cell Line

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A- PRODUCT DESCRIPTION

Recombinant CHO-K1 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:** CHO-K1.
- **Selection marker:** G418 1.2 mg/ml.
- **Biosafety level:** 1.
- **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

F-12 Nutrient Mixture (Ham) (eg. Invitrogen 21765-029)

10% Foetal Bovine Serum (FBS) (eg. PAA ref. 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 ref. 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. Grow cells in a humidified incubator at 37°C under 5% carbon dioxide.
5. All 3-4 days, dilute cells. To maintain electrophysiological performances, cell density must not exceed 80%.

C4 • Protocol - Passaging adherent cells

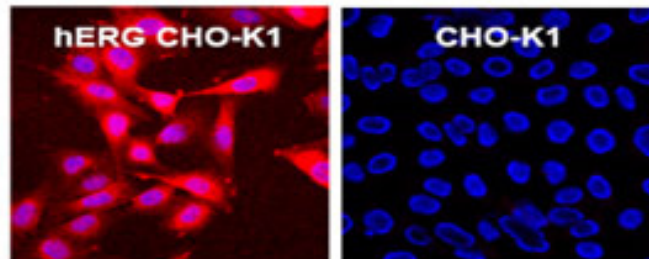
1. Remove poor medium and rinse the cells once with PBS1X.
2. Add 1-2 ml of Trypsin-EDTA solution. Place plate on a 37°C warming incubator 3 to 5 min. As soon as cells are detached, add 9 ml of 37°C complete medium. Draw cell suspension into a sterile pipet and homogenize cells gently to dissociate cells aggregates.
3. Count cells using a hemacytometer with Blue Trypan. Centrifuge 5 min at 400 g.
4. Amplify or maintain the cells by seeding $2 \cdot 10^5$ cells/ml in a T75 flask (final volume: 15 ml).

C5 • Protocol - Freezing cells

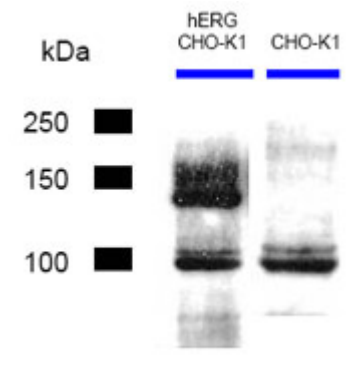
1. Trypsinize cells (see C4, steps 1 to 3).
2. Remove supernatant and add 1 ml of freezing medium (FBS 90%, DMSO 10%). Resuspend pellet. Dilute with freezing medium as necessary to get a final cells concentration of $5 \cdot 10^6$ cells/ml.
3. Transfer 1-ml aliquots of cell suspension into labeled 2-ml 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 hERG CHO-K1 cells or wild type CHO-K1 cells with anti-hERG antibody (red). Nucleus staining is performed with Hoechst (blue).



D2 • Western blot analysis of recombinant or wild type CHO-K1 total cell extracts with anti-hERG antibody.



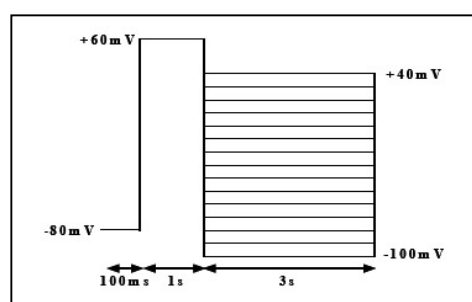
E- PATCH CLAMP VALIDATION

E1 • Solutions:

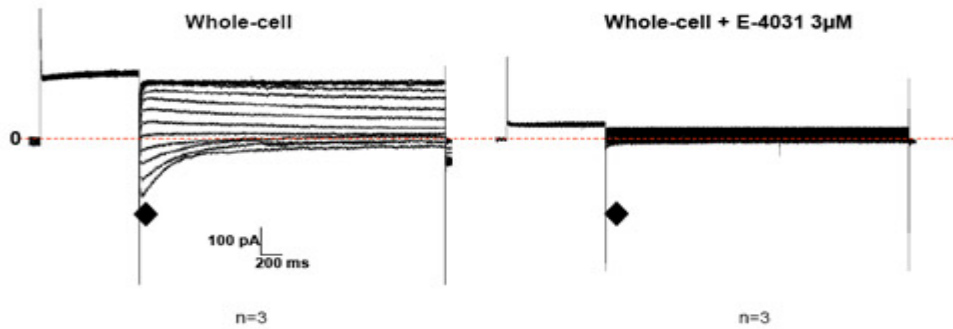
- Extracellular solution: 118 mM NaCl, 5.6 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgCl₂, 10 mM HEPES, 11 mM glucose, pH 7.4
- Intracellular pipet solution: 30 mM KCl, 110 mM K-aspartate, 0.1 mM CaCl₂, 1 mM MgCl₂, 1 mM EGTA, 10 mM HEPES, pH=7.3
- E-4031: SIGMA M5060.

E2 • Configuration: whole-cell.

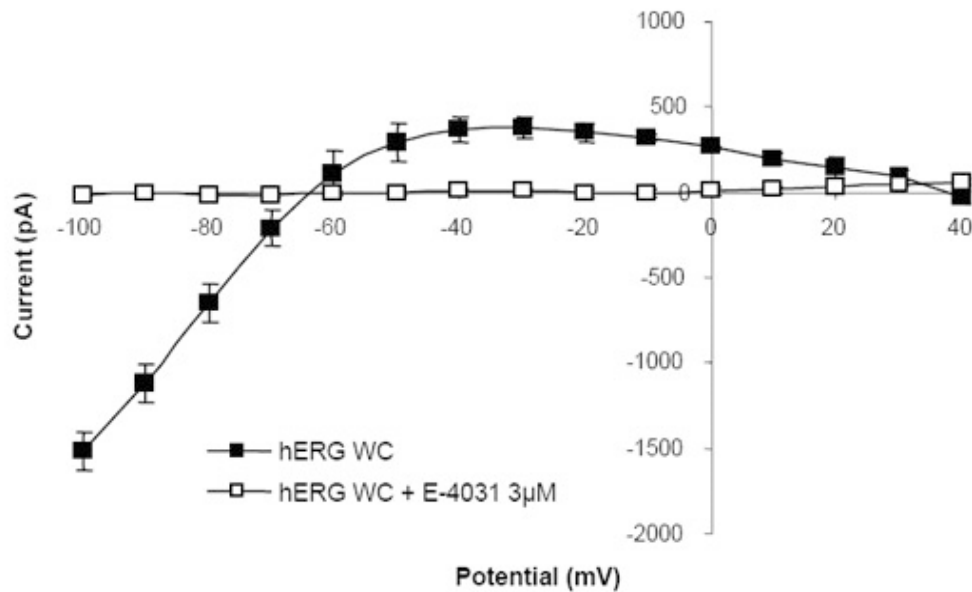
E3 • Recording procedure:



E4 • Currents recorded from hERG CHO-K1 recombinant cell line:



E5 • I-V curves - E-4031 (3 μ M):



E6 • Current stability over continuous culture.

The hERG CHO-K1 cell line was propagated over 3 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 CHO-K1 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 CHO-K1 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 CHO-K1 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 CHO-K1 cell line, or to any part thereof, to Purchaser.

■ CREACELL warrants that it has the right to supply the CreaCell™ hERG CHO-K1 cell line and to provide the License to the Purchaser, and that the Purchaser and its Affiliate's use of the CreaCell™ hERG CHO-K1 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 CHO-K1 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 CHO-K1 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).