

CreaCell™ hKir2.1 HEK293 Cell Line

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A- PRODUCT DESCRIPTION

Recombinant HEK293 cell line expressing the human Kir2.1 potassium channel:

- **Expressed recombinant protein:** Homo sapiens potassium inwardly-rectifying channel, subfamily J, member 2 (KCNJ2), mRNA. cDNA strictly similar to GenBank accession number: NM_000891.
- **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 21769029)

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

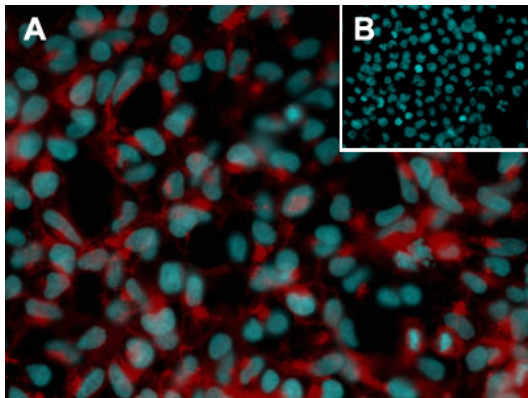
1. Remove poor medium and rinse the cells once with PBS1X.
2. Add 1-2 ml of Trypsin-EDTA solution. Place plate on a 37°C warming incubator 3 to 5 min. As soon as cells are detached, add 9 ml of 37°C complete medium. Draw cell suspension into a sterile pipet and homogenize cells gently to dissociate cells 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 wild type HEK293 cells (B) or hKir2.1 HEK293 cells (A). Detection with anti-hkir2.1 antibody (red) and nucleus staining performed with Hoechst (blue).



E- PATCH CLAMP VALIDATION

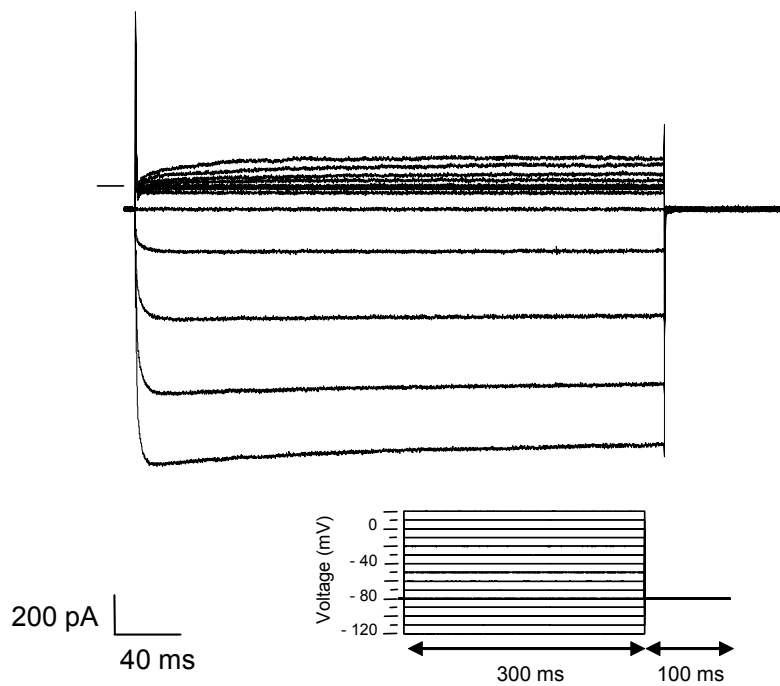
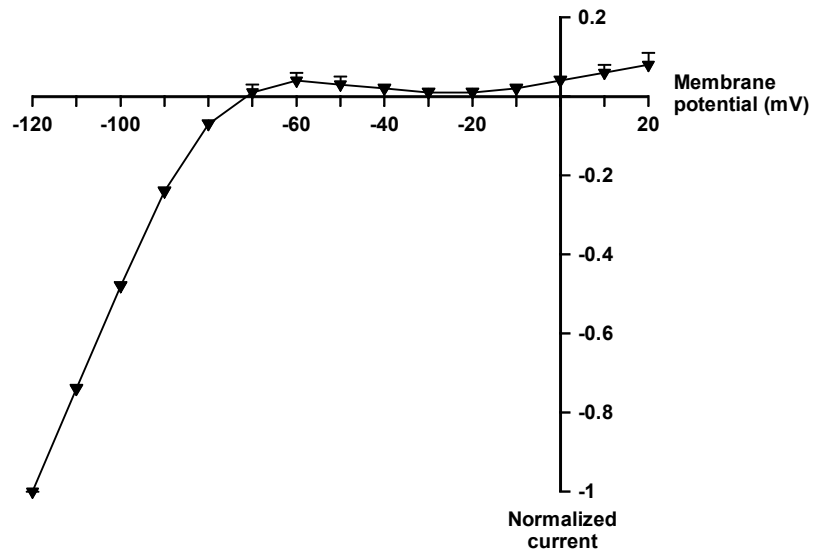
E1 • Solutions.

- Intra-pipette solution : 130 mM Potassium aspartate, 5 mM MgCl₂, 4 mM ATP, 10 mM HEPES, 10 mM EGTA, pH of 7.20 ± 0.05 adjusted with KOH.
- Extra-cellular solution (vehicle control used for perfusing test/reference substances - mM): 145 mM NaCl, 4.5 mM KCl, 10 mM HEPES, 5 mM glucose, 1.5 mM CaCl₂: 1.5, 1 mM MgCl₂, pH of 7.35 ± 0.05 adjusted with NaOH.

E2 • Configuration: whole-cell. Current measurements were normalized using the cell capacitance as an index of cell surface (average for 3 consecutive stimuli). Experimental bath temperature: 19.3– 21 °C.

E3 • Voltage dependent properties of hKir2.1 HEK293.

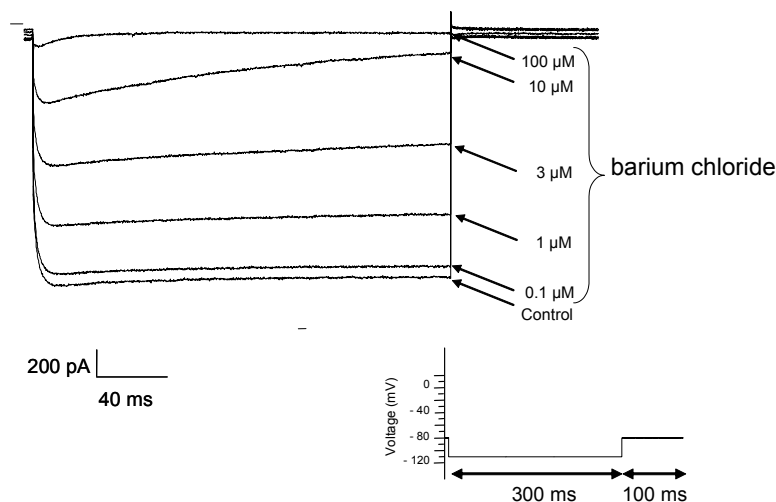
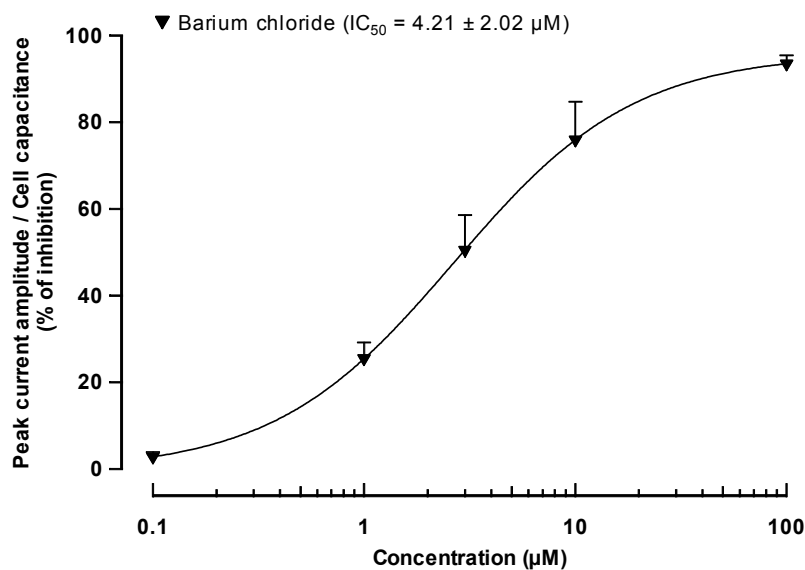
Currents were elicited by applying 300 ms pulses from -120 to +20 mV in 10 mV increments from a holding potential of -80 mV at a stimulation frequency of 1 Hz. Current amplitude (pA) was measured at the end of each 300 ms-pulse. Current-voltage (IV) curves were obtained from each cell and then averaged (mean ± s.e.m.). Three cells were studied.



hKir2.1 HEK293 cell line shows the expected voltage-dependent properties of the inward rectifier Potassium current (I_{K1}). The current displayed inward rectification with reversal at -60 mV.

E4 • Pharmacological validation of hKir2.1 HEK293 with barium chloride.

Cells were stimulated every second (1 Hz) using the following protocol: 300 ms pulse to -110 mV from a holding potential of -80 mV during which current was measured (current amplitude measured at the end of the 300 ms-pulse). Effect of barium chloride was studied at 0.1, 1, 3, 10 and 100 μM in order to establish individual concentration-response curves. For each concentration, the effects were reported after steady state on I_{K1} current was reached. IC_{50} values were estimated from each individual curve and then averaged (mean \pm sem). Four cells were studied.



hKir2.1 HEK293 cell line shows satisfying sensitivity to the inhibiting effects of barium chloride with estimated IC_{50} value of $4.21 \pm 2.02 \mu\text{M}$.

E5 • Current stability over continuous culture.

The hKir2.1 HEK293 cell line was propagated over 4 months under the recommended growth conditions. There was no significant variation in expression of the hKir2.1 current over this period.

F- LICENSE AGREEMENT

(Extract)

■ The CreaCell™ hKir2.1 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™ hKir2.1 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™ hKir2.1 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™ hKir2.1 HEK293 cell line, or to any part thereof, to Purchaser.

■ CREACELL warrants that it has the right to supply the CreaCell™ hKir2.1 HEK293 cell line and to provide the License to the Purchaser, and that the Purchaser and its Affiliate's use of the CreaCell™ hKir2.1 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™ hKir2.1 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™ hKir2.1 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).