

CreaCell™ hNav1.5 HEK293 Cell Line

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A- PRODUCT DESCRIPTION

Recombinant HEK293 cell line expressing the human Nav1.5 sodium channel:

- **Expressed recombinant protein:** Homo sapiens sodium channel, voltage-gated, type V, alpha subunit (SCN5A), transcript variant 2, mRNA. cDNA strictly similar to GenBank accession number: NM_000335.
- **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 geneticin 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).

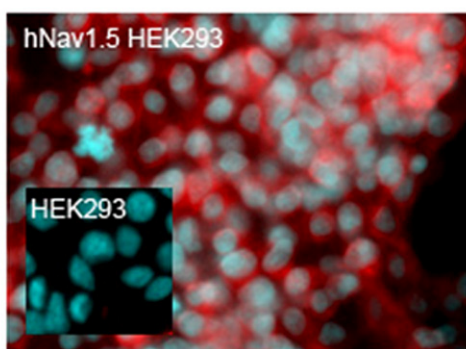
C5 • Protocol - Freezing cells

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.

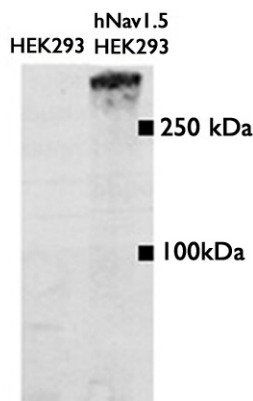
D- BIOCHEMICAL VALIDATION

D1 • Immunofluorescence & western blot of wild type HEK293 cells or hNav1.5 HEK293 cells. Immunofluorescence: detection with anti-hNav1.5 antibody (red) and nucleus staining performed with Hoechst (blue).

Immunofluorescence



Western blot



E- PATCH CLAMP VALIDATION

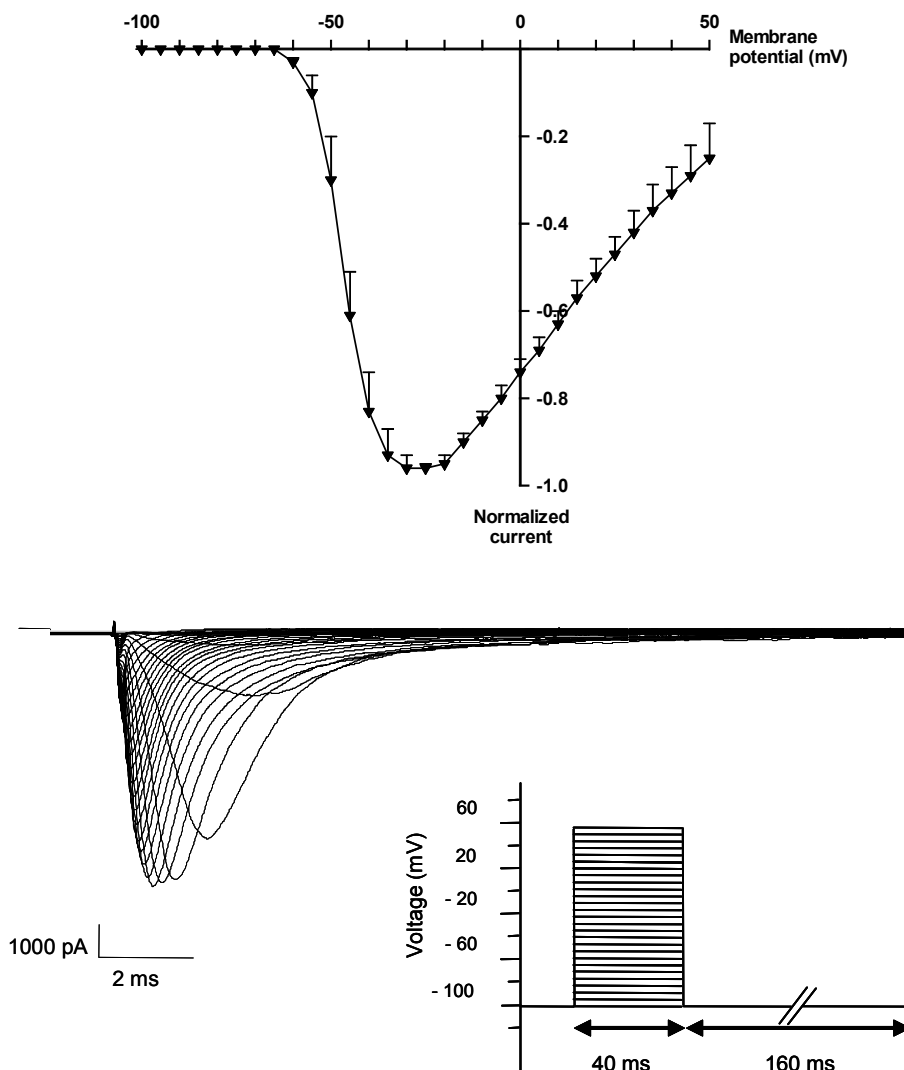
E1 • Solutions:

- Intra-pipette solution : 10 mM NaF, 110 mM CsF, 20 mM CsCl, 10 mM EGTA; 10 mM HEPES, pH of 7.35 ± 0.05 adjusted with CsOH.
- 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 CaCl_2 , 1 mM MgCl_2 , pH of 7.35 ± 0.05 adjusted with CsOH.

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: 18.0 – 22.9 °C.

E3 • Voltage dependent properties of hNav1.5 HEK293.

Currents were elicited by applying 40 ms pulses from -100 to +50 mV in 5 mV increments from a holding potential of -100 mV at a stimulation rate of 1 Hz. Peak current amplitude (pA) was measured at each test potential. Current-voltage (IV) curves were obtained from each cell (seven cells were studied) and then averaged (mean \pm s.e.m.).

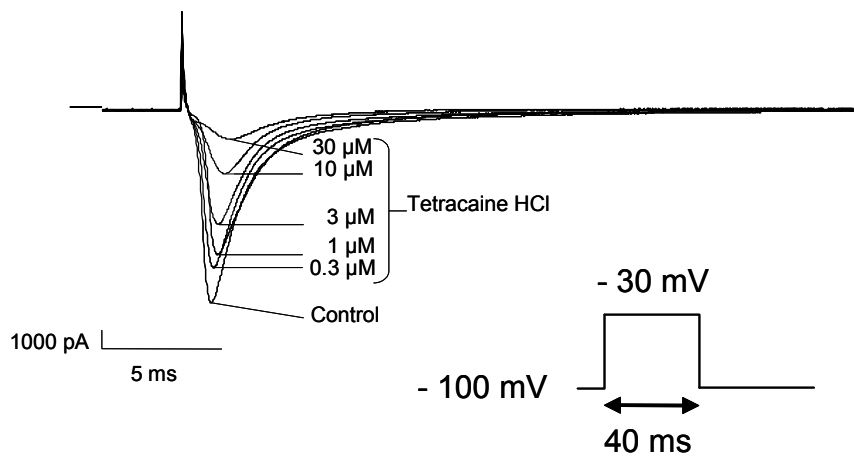


hNav1.5 HEK293 shows the expected voltage-dependent properties of type 1 sodium current, with estimated threshold potential of -60 mV and estimated potential inducing the maximal amplitude of I_{Na} of -25 mV, respectively.

E4 • Pharmacological validation of hNav1.5 HEK293 with Tetracaine or Lidocaine.

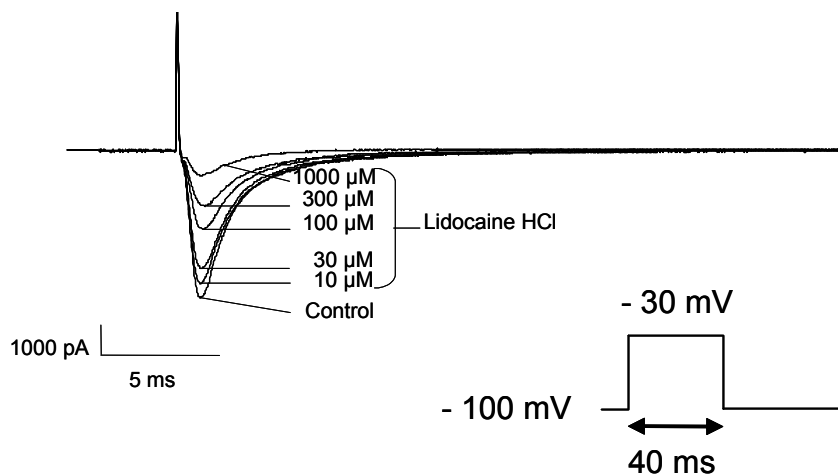
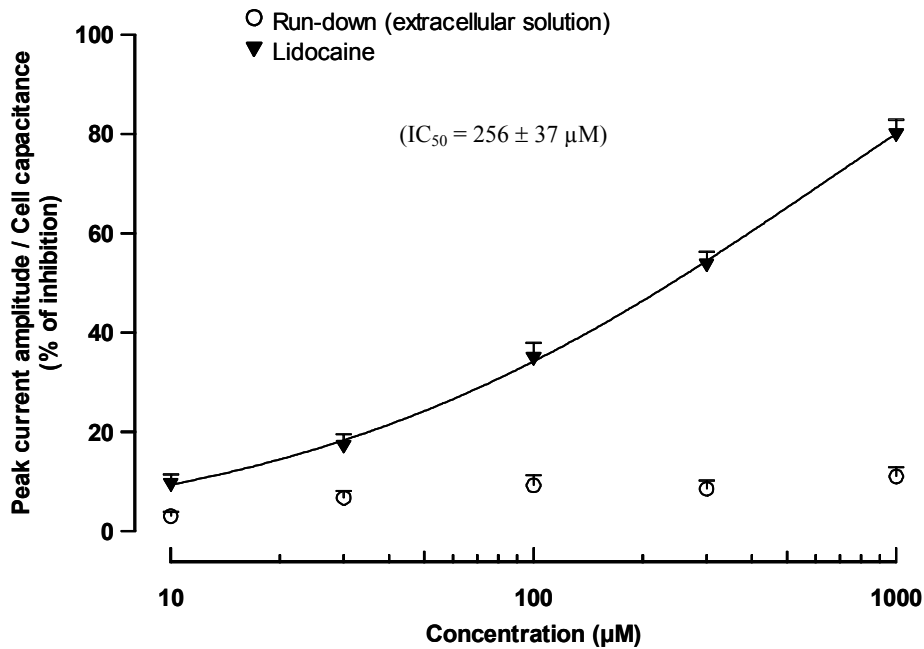
Cells were stimulated every second (1 Hz) using the following protocol: 40 ms pulse to -30 mV from a holding potential of -100 mV during which peak current is measured. Effects of Tetracaine (0.3, 1, 3, 10 and 30 μ M) or Lidocaine (10, 30, 100, 300 and 1000 μ M) were studied to establish individual concentration-response curves. For each concentration, the effects were reported after steady state on I_{Na} current was reached. IC_{50} values were estimated from each individual curve and then averaged (mean \pm s.e.m.). Six (tetracaine) and 12 (lidocaine) cells were studied.

■ Tetracaine



hNav1.5 HEK293 shows negligible run-down of INa current under control conditions and satisfying sensitivity to the inhibiting effects of Tetracaine with estimated IC_{50} of $2.61 \pm 0.72 \mu M$.

■ Lidocaine



hNav1.5 HEK293 shows negligible run-down of INa current under control conditions and satisfying sensitivity to the inhibiting effects of Lidocaine with estimated IC₅₀ of 256 ± 37 µM.

E5 • Current stability over continuous culture.

The hNav1.5 HEK293 recombinant cell line was propagated over 4 months under the recommended growth conditions. There was no significant variation in expression of the hNav1.5 current over this period.

F- LICENSE AGREEMENT

(Extract)

■ The CreaCell™ hNav1.5 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™ hNav1.5 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™ hNav1.5 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™ hNav1.5 HEK293 cell line, or to any part thereof, to Purchaser.

■ CREACELL warrants that it has the right to supply the CreaCell™ hNav1.5 HEK293 cell line and to provide the License to the Purchaser, and that the Purchaser and its Affiliate's use of the CreaCell™ hNav1.5 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™ hNav1.5 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™ hNav1.5 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).