



Stably Transfected Cell Line Data Sheet
hHCN4-HEK
Catalog Number CT6006

Related Services and Products

FastPatch[®] and ScreenPatch[™] automated patch clamp services
Replicating hHCN4-CHO cell line. Cat. No. CT6177.
EZCell[™]-DA. Division-arrested cell line hHCN4-CHO. Cat. No. CT4177.
Additional information available at www.chantest.com

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Table of Contents

1	Cell Line Description.....	3
1.1	Background.....	3
1.2	Pore-forming subunit identifier: hHCN4.....	3
1.3	Sequence Information.....	3
1.4	Expression System.....	3
1.5	Product Format.....	3
1.6	Mycoplasma Status: Negative.....	3
1.7	Cell line Stability.....	3
2	Validated Test Platforms.....	4
2.1	Representative Manual Patch Clamp Data.....	4
2.2	PatchXpress®.....	5
2.2.1	Throughput Capability in PatchXpress®.....	5
2.2.2	Representative Data.....	6
3	References.....	6

1 Cell Line Description

1.1 Background

HCN4 is a hyperpolarization-gated, cyclic nucleotide-sensitive, non-selective cation channel. HCN4 channels expressed in cardiac and neuronal cells regulate pacemaking currents (I_h).

1.2 Pore-forming subunit identifier: hHCN4

Class: Hyperpolarization-gated channel

Species: Human

Gene name: HCN4

1.3 Sequence Information

The cDNA sequence of the HCN4 gene used to create this cell line was confirmed prior to transfection. The amino acid sequence encoded by the transfected cDNA is identical to the translated sequence for GenBank accession number NM_005477.1.

1.4 Expression System

HEK293 (human embryonic kidney cells), tetracycline-inducible expression.

1.5 Product Format

Cryopreserved cells, 1×10^6 cells/vial

1.6 Mycoplasma Status: Negative

The absence of mycoplasma species in this cell line was confirmed with the MycoAlert Kit (Lonza Rockland, Inc.).

1.7 Cell line Stability

Table 1. Stability of hHCN4 Current

Passage Number	Current Amplitude (pA)	n
29	1.27 ± 0.20	13
33	2.07 ± 0.63	10
41	2.09 ± 0.53	13
49	1.27 ± 0.29	14
57	2.22 ± 0.61	6

* cells incubated ~ 24 hours at 27 °C prior to recording

A frozen vial at P24 was thawed and passaged for stability measurements. hHCN4 current amplitudes recorded by PatchXpress[®] (mean \pm standard deviation). Table 1 shows that current amplitude measured from hHCN4-HEK remains stable for at least 33 passages beyond the original frozen vial.

2 Validated Test Platforms

Electrophysiological and pharmacological verification of the functional properties of the cloned channels was assessed in the following test platforms:

Manual Patch Clamp
PatchXpress[®] (MDS-AT)

2.1 Representative Manual Patch Clamp Data

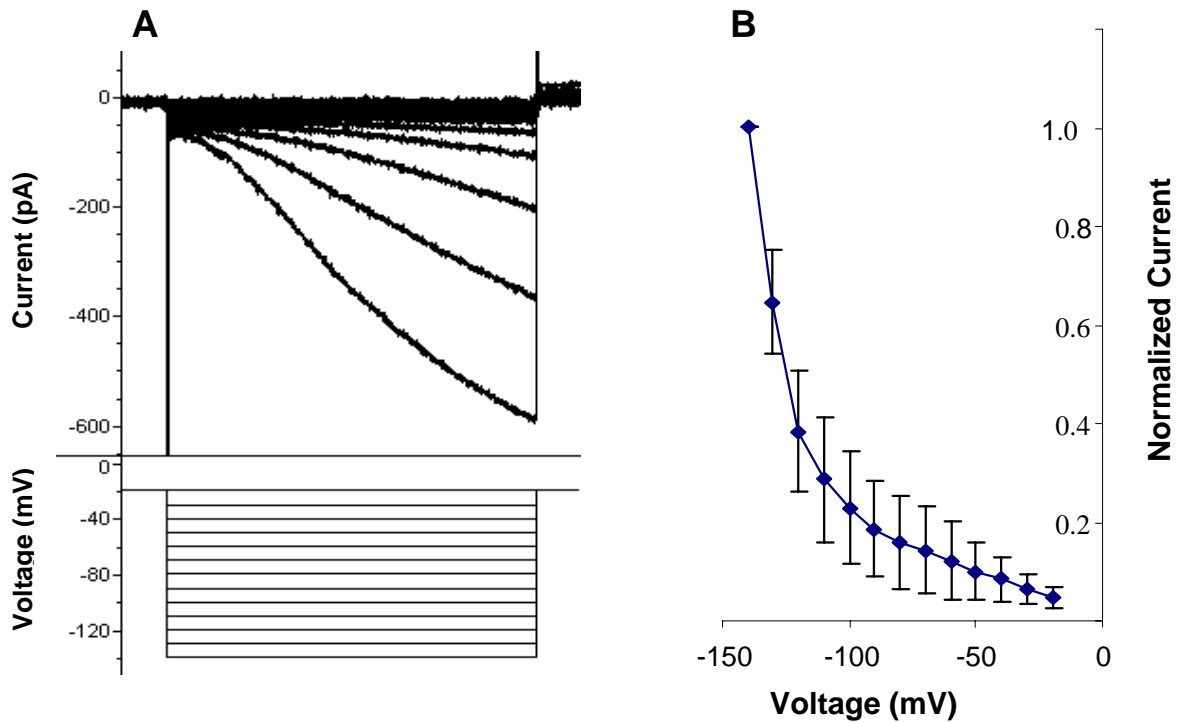


Figure 1. Hyperpolarization-Dependent Gating in Manual Patch Clamp

A: Family of traces (upper panel) elicited by hyperpolarizing test steps (lower panel).

B: Current-voltage relationship measured at the end of the hyperpolarizing step (Mean \pm SEM, n = 7 cells).

2.2 PatchXpress®

2.2.1 Throughput Capability in PatchXpress®

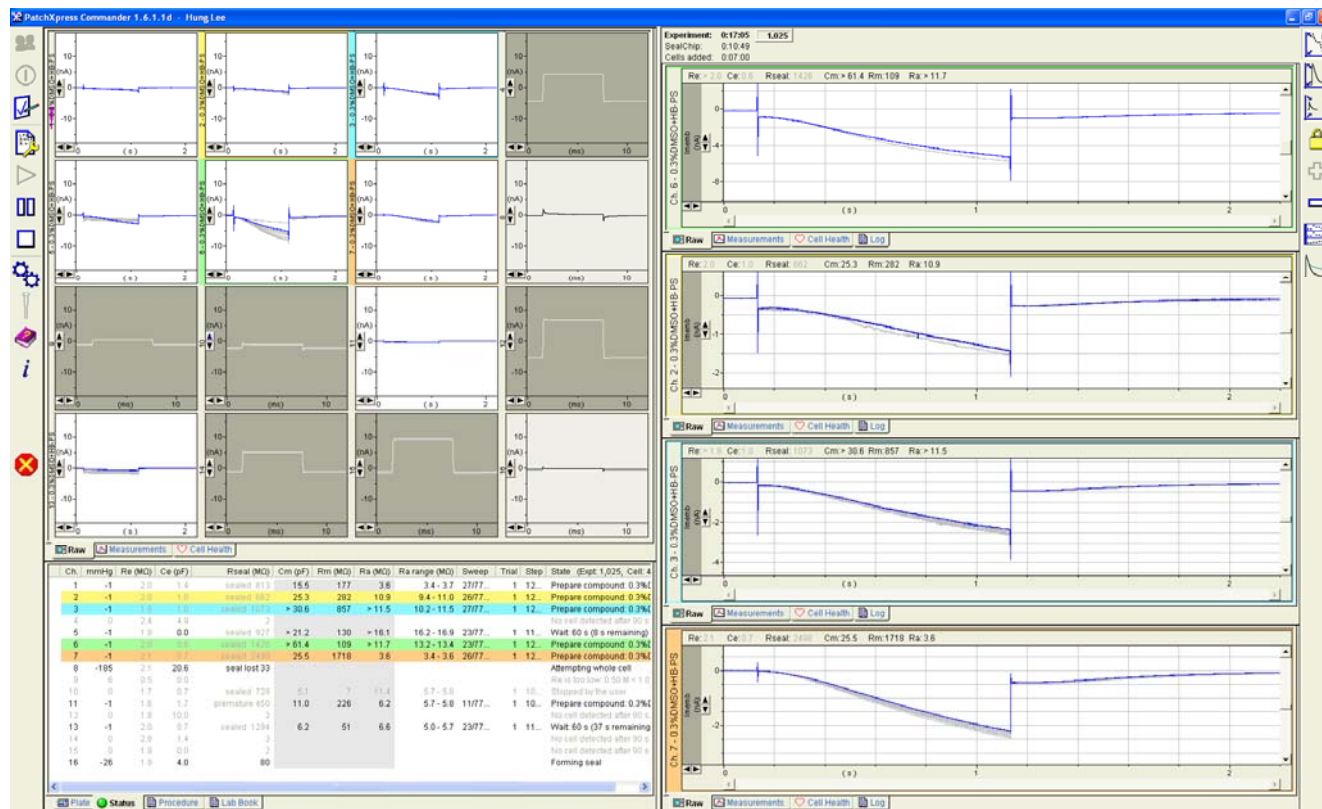


Figure 2. PatchXpress® Throughput Capability

Throughput in PatchXpress® depends upon many factors that introduce variability in success rates. The screen capture (Figure 2) shows a typical PatchXpress® experiment. In this example, cells were dispensed into 15 of 16 wells, 10 seals formed, of a possible 16 seals were formed, whole-cell configuration was achieved in eight cells, and six cells showed characteristic hHCN4 current waveforms with very low leak current and peak current amplitudes >1 nA.

2.2.2 Representative Data

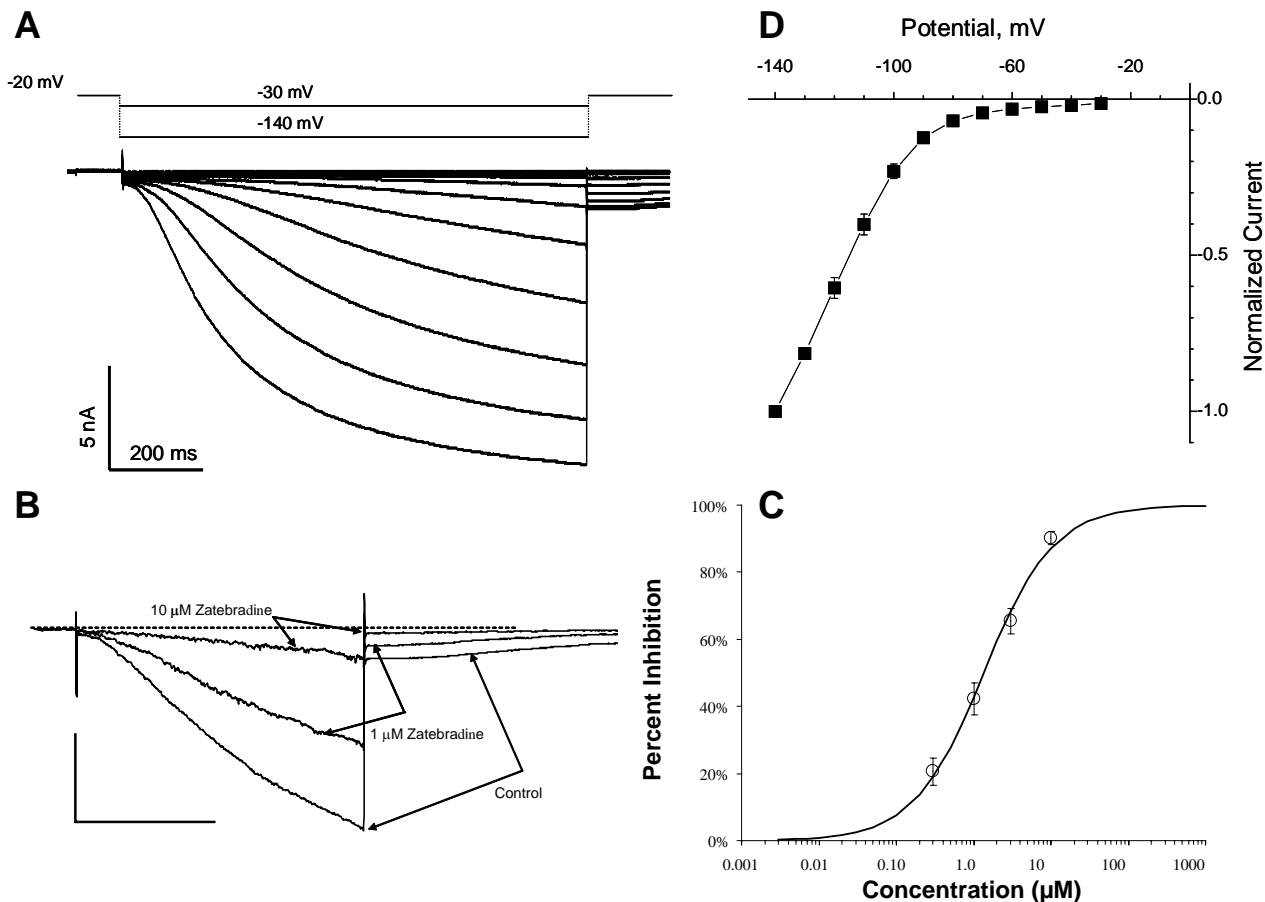


Figure 3. Voltage-Dependent Gating and Zatebradine Block.

A: Hyperpolarization-dependent activation. Currents were elicited by test pulses from -30 to -140 mV in 10 mV decrements from holding potential, -30 mV.
B: Current-voltage relationship. **C:** Effect of 1 and 10 μM zatebradine on hHCN4 currents evoked by test potentials to -120 mV. Horizontal and vertical calibrations are 0.5 s and 0.5 nA, respectively. **D:** Zatebradine concentration-response relationship (Mean \pm SEM, n = 5 - 7 cells/concentration). IC_{50} = 1.4 μM .

3 References

Hofmann F, et al. 2005. *Pharmacol Rev.* 57:455 - 462. International Union of Pharmacology. LI. Nomenclature and molecular relationships of cyclic nucleotide-regulated channels.