Stably Transfected Cell Line - Product Data Sheet

$hK_v4.3$-HEK

Catalog Number CT6144

Related Services and Products

FastPatch® and ScreenPatch™ automated patch clamp services
Replicating $hK_v4.3$-CHO cell line. Cat. No. CT6143
Replicating $hK_v4.3$/KChIP2.2-CHO cell line. Cat. No. CT6171
Additional information available at www.chantest.com

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1 Cell Line Description

1.1 Background

K_v4.3 is a voltage-gated, K^+-selective channel expressed in the heart, central nervous system, and smooth muscle. In heart, K_v4.3 mediates a transient outward current, I_{to}, responsible for phase 1 action potential repolarization. The channel is a potential therapeutic target in atrial fibrillation.

1.2 Pore-forming subunit identifier: hK$_v$4.3

Class: Voltage-gated potassium channel
Species: Human
Gene name: KCND3

1.3 Sequence Information

The cDNA sequence of the KCND3 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_004980.3.

1.4 Expression System

HEK293 (human embryonic kidney) cells, constitutive expression.

1.5 Product Format

Cryopreserved cells, 1 x10^6 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 hK$_v$4.3 Current

Passage Number	Current Amplitude (nA)	n (cells)
27	1.61 ± 0.37	10
31	5.50 ± 2.37	4
39	4.01 ± 2.30	8
47	3.31 ± 0.76	15
55	2.73 ± 0.69	18

hK$_v$4.3 currents recorded by PatchXpress® (Mean ± SEM). A frozen vial at P22 was thawed and passaged for stability measurements. Current amplitudes remained stable for at least 33 passages beyond P22.

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 Manual Patch Clamp Representative Data

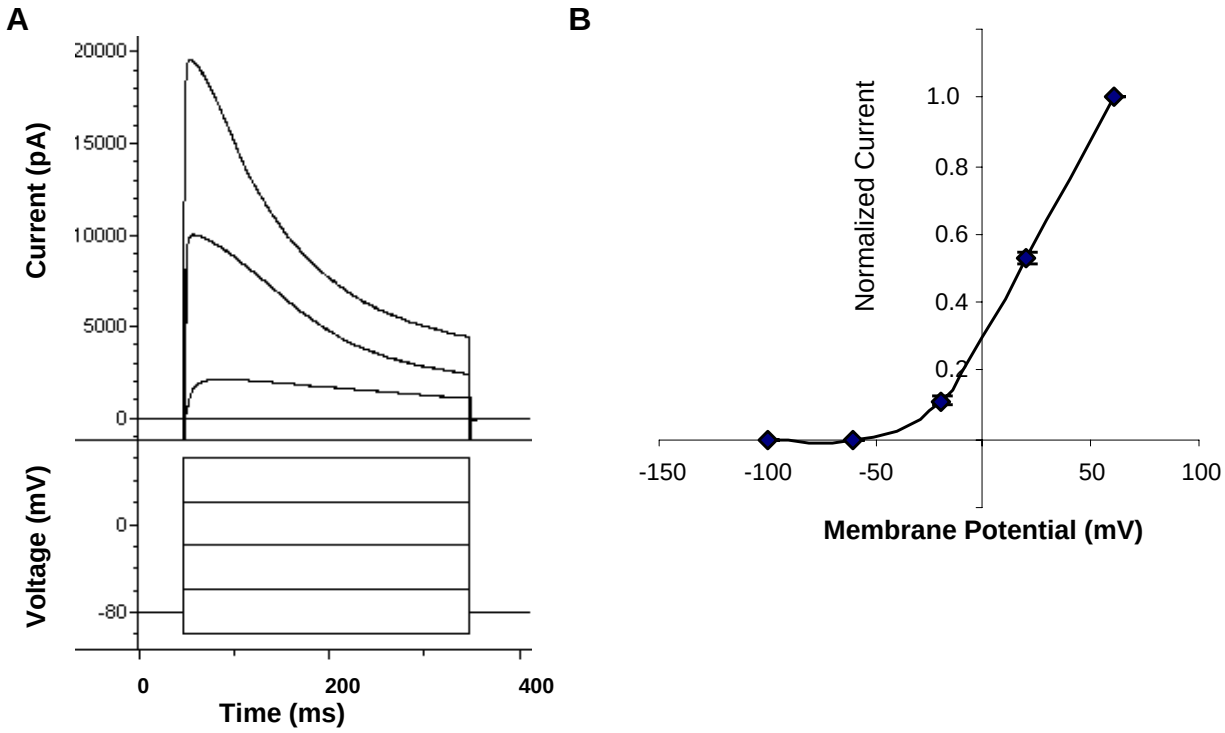


Figure 1. Voltage-Dependent $hK_v4.3$ Gating in Manual Patch Clamp.
A: Family of current traces (upper panel) elicited by a voltage pattern (lower panel) of test pulses ranging from -100 to +60 mV, in 40 mV increments holding potential -80 mV. **B:** Peak current-voltage relationship.

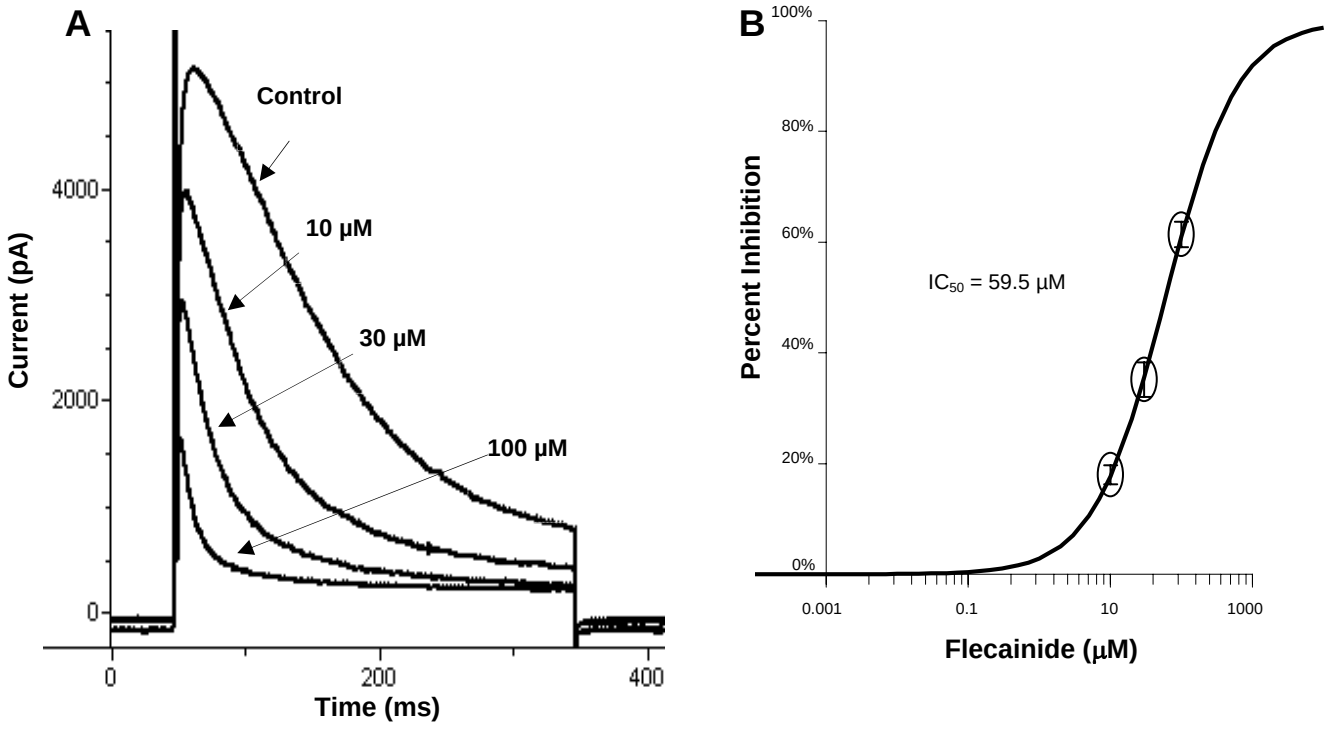
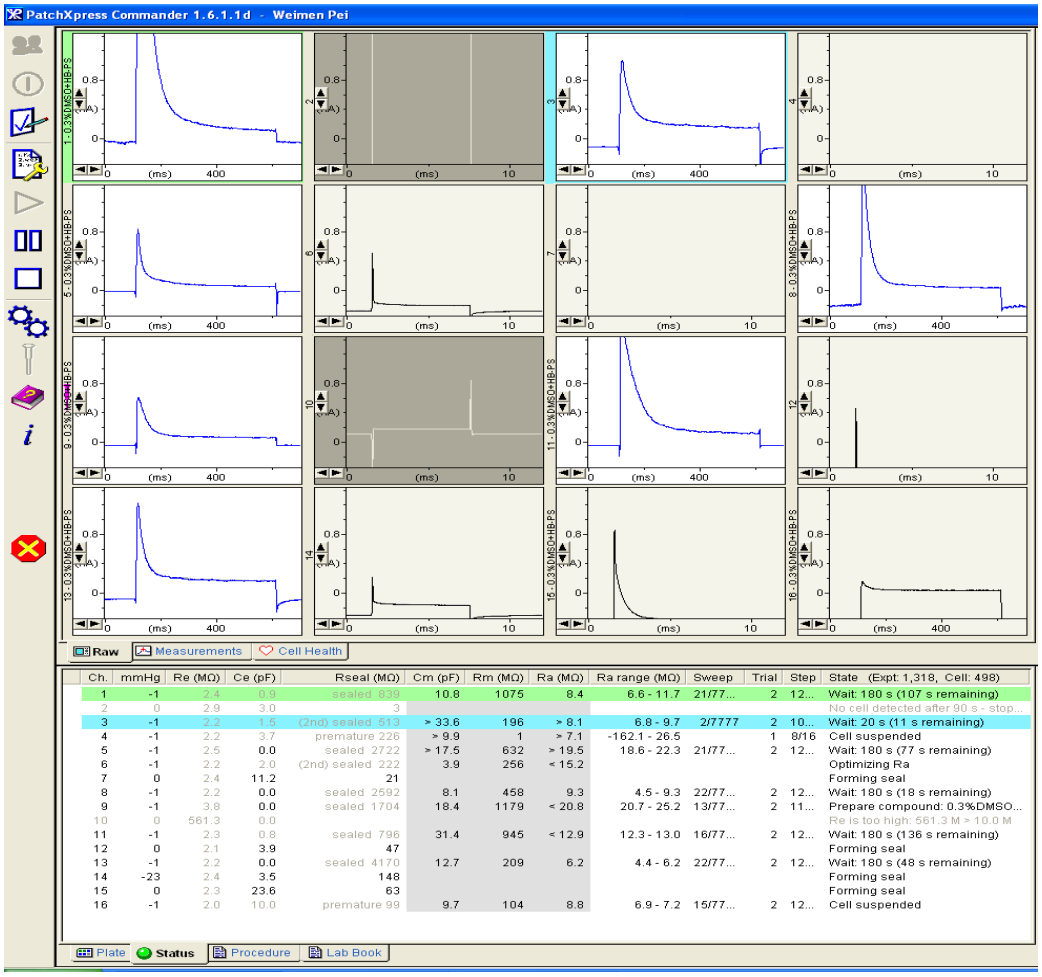


Figure 2. Flecainide Block in Manual Patch Clamp
A: Current traces elicited by test pulses to 0 mV, holding potential -80 mV.
B: Concentration-response relationship (Mean peak current amplitude ± SEM, n = 3 - 4 cells/concentration). IC_{50} = 59.5 µM.

2.2 PatchXpress®

2.2.1 Throughput Capability in PatchXpress®



Ch.	mmHg	Re (MΩ)	Ce (pF)	Rseal (MΩ)	Cm (pF)	Rm (MΩ)	Ra (MΩ)	Ra range (MΩ)	Sweep	Trial	Step	State (Expt: 1,318, Cell: 498)
1	-1	2.4	0.9	sealed 839	10.8	1075	8.4	6.6 - 11.7	21/77...	2	12...	Wait 180 s (107 s remaining)
2	0	2.9	3.0	3								No cell detected after 90 s - stop...
3	-1	2.2	1.5	(2nd) sealed 513	> 33.6	196	> 8.1	6.8 - 9.7	2/7777	2	10...	Wait 20 s (11 s remaining)
4	-1	2.2	3.7	premature 226	> 9.9	1	> 7.1	-162.1 - 26.5		1	8/16	Cell suspended
5	-1	2.5	0.0	sealed 2722	> 17.5	632	> 19.5	18.6 - 22.3	21/77...	2	12...	Wait 180 s (77 s remaining)
6	-1	2.2	2.0	(2nd) sealed 222	3.9	256	< 15.2					Optimizing Ra
7	0	2.4	11.2	21								Forming seal
8	-1	2.2	0.0	sealed 2592	8.1	458	9.3	4.5 - 9.3	22/77...	2	12...	Wait 180 s (18 s remaining)
9	-1	3.8	0.0	sealed 1704	18.4	1179	< 20.8	20.7 - 25.2	13/77...	2	11...	Prepare compound: 0.3%DMSO...
10	0	561.3	0.0									Re is too high: 561.3 M > 10.0 M
11	-1	2.3	0.8	sealed 796	31.4	945	< 12.9	12.3 - 13.0	16/77...	2	12...	Wait 180 s (136 s remaining)
12	0	2.1	3.9	47								Forming seal
13	-1	2.2	0.0	sealed 4170	12.7	209	6.2	4.4 - 6.2	22/77...	2	12...	Wait 180 s (48 s remaining)
14	-23	2.4	3.5	148								Forming seal
15	0	2.3	23.6	63								Forming seal
16	-1	2.0	10.0	premature 99	9.7	104	8.8	6.9 - 7.2	15/77...	2	12...	Cell suspended

Figure 3. PatchXpress® hKv4.3-HEK Screen Capture.
Throughput capability in PatchXpress® depends upon many factors which may result in success rate variability. The screen capture shows a typical hKv4.3 PatchXpress® experiment: 8 of a possible 16 seals were formed, whole-cell configuration was achieved in 8 cells, and 7 cells showed characteristic hKv4.3 current waveforms with little leak current and peak current amplitudes > 0.6 nA.

2.2.2 Representative PatchXpress® Data

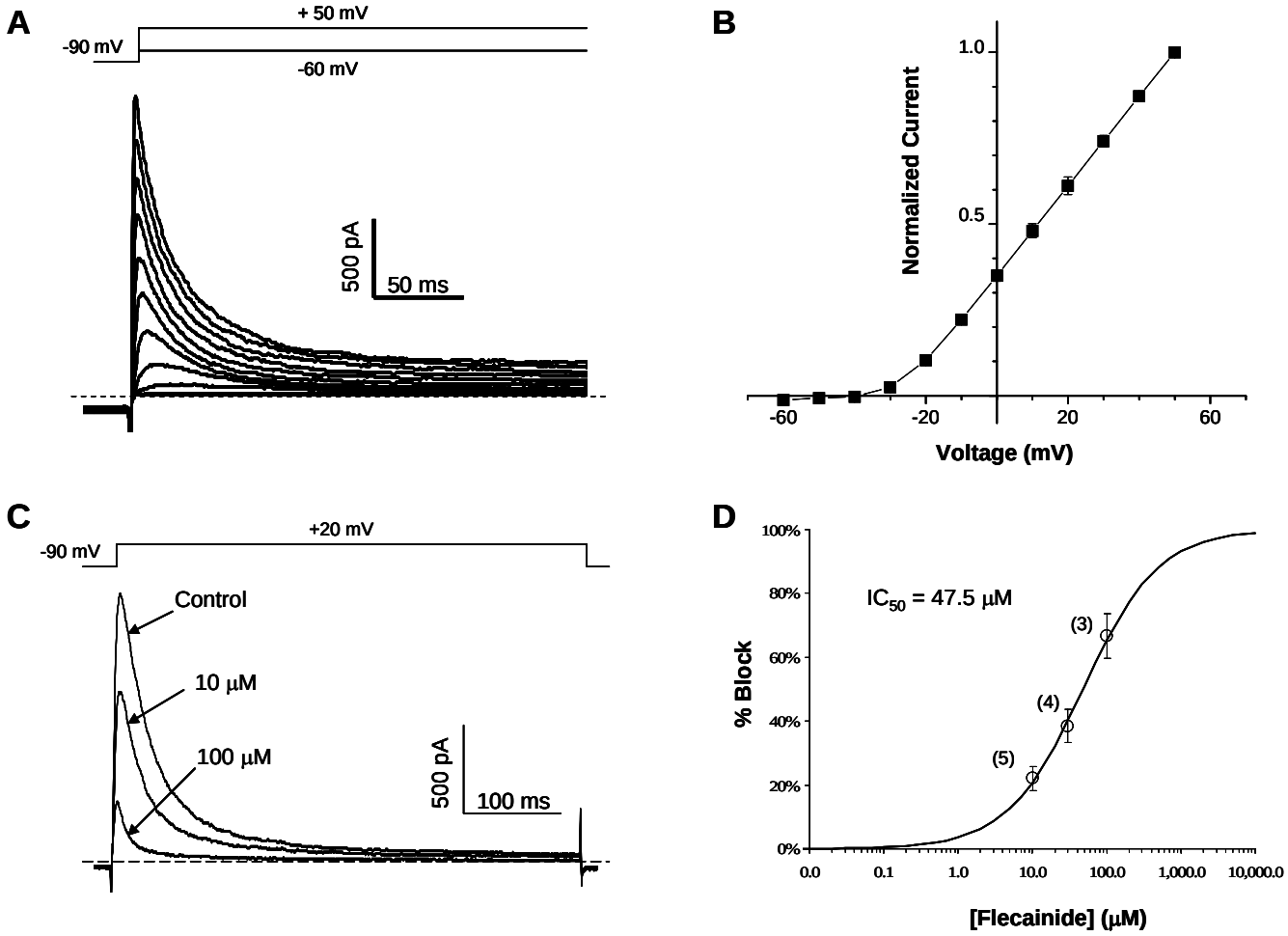


Figure 4. Voltage-dependent Gating and Flecainide Block in PatchXpress®
A: Current traces elicited by voltage steps -60 to +50 in 10 mV increments, holding potential, -90 mV. **B:** Current-voltage relationship. Peak current amplitudes normalized to maximum at +50 mV. **C**: Concentration-dependent block by flecainide. Currents elicited by test pulses to + 20 mV. **D:** Concentration-response relationship (Mean peak current amplitude ± SEM, n = 3 - 5 cells/concentration). IC_{50} = 47.5 µM).

3 References

Gutman GA, et al. 2005. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. Pharmacol Rev. 57:473-508.