



Stably Transfected Cell Line - Product Data Sheet
hK_v7.4-CHO
Catalog Number CT6016

Related Services and Products

FastPatch[®] and ScreenPatch[™] automated patch clamp services
EZCell[®] DA, division-arrested hK_v7.4-CHO cells. Cat. no. CT4171
Additional information available at www.chantest.com

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1 Cell Line Description

1.1 Background

K_v7.4 is a voltage-gated, K⁺-selective channel that regulates neuronal excitability. K_v7.4 channels are potential therapeutic targets in epilepsy.

1.2 Pore-forming subunit identifier: hK_v7.4

Class: Voltage-gated potassium channel
Species: Human
Gene Name: KCNQ4

1.3 Sequence Information

The cDNA sequence of the KCNQ4 gene used to create this cell line was confirmed prior to transfection. The amino acid sequence encoded by the transfected cDNAs was identical to the translated sequence for GenBank accession number NM_004700.2.

1.4 Expression System

CHO (Chinese hamster ovary) cells, constitutive expression.

1.5 Product Format

Cryopreserved cells, 1 x10⁶ 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.) performed at ChanTest and a DNA fluorescence assay performed at Bionique Testing Laboratories, Inc.

1.7 Cell Line Stability

Channel expression in this cell line has been shown to be stable (current amplitude ≥ 230 pA at +20 mV) for at least 42 passages.

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[®]
QPatch[™] HT (Sophion)
FLIPR[®] (MDS-AT)

2.1 Manual Patch Clamp Representative Data

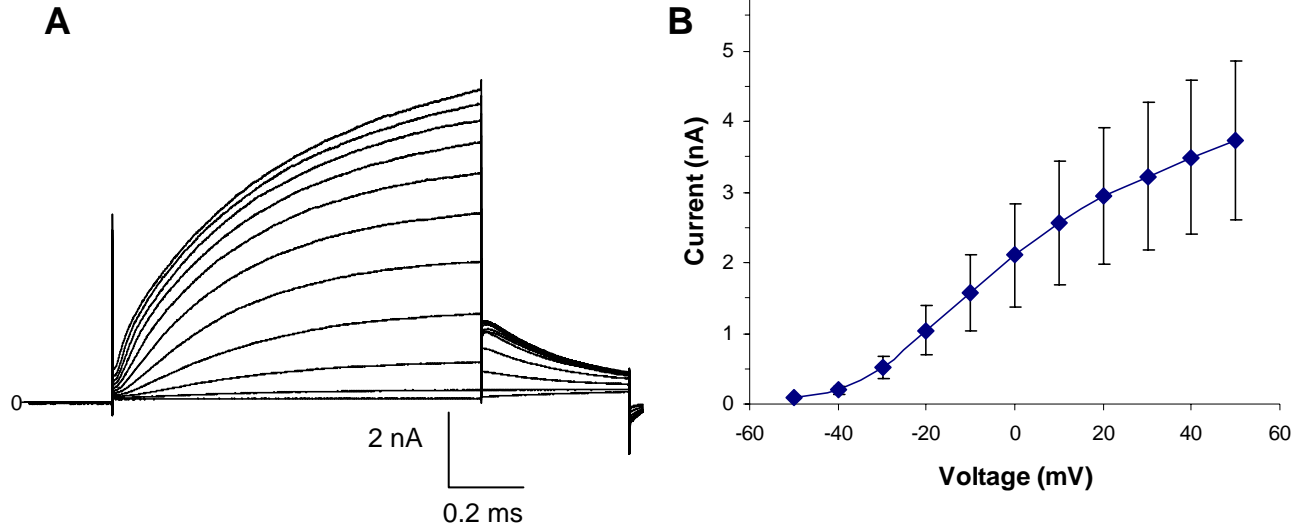


Figure 1. Voltage-Dependent Kv7.4 Activation in Manual Patch Clamp

A: Currents elicited by test pulses ranging from -50 to +50 mV in 10 mV increments, return potential, -40 mV; holding potential -80 mV. **B:** Current-voltage relationship. Mean \pm SEM, n = 5 cells.

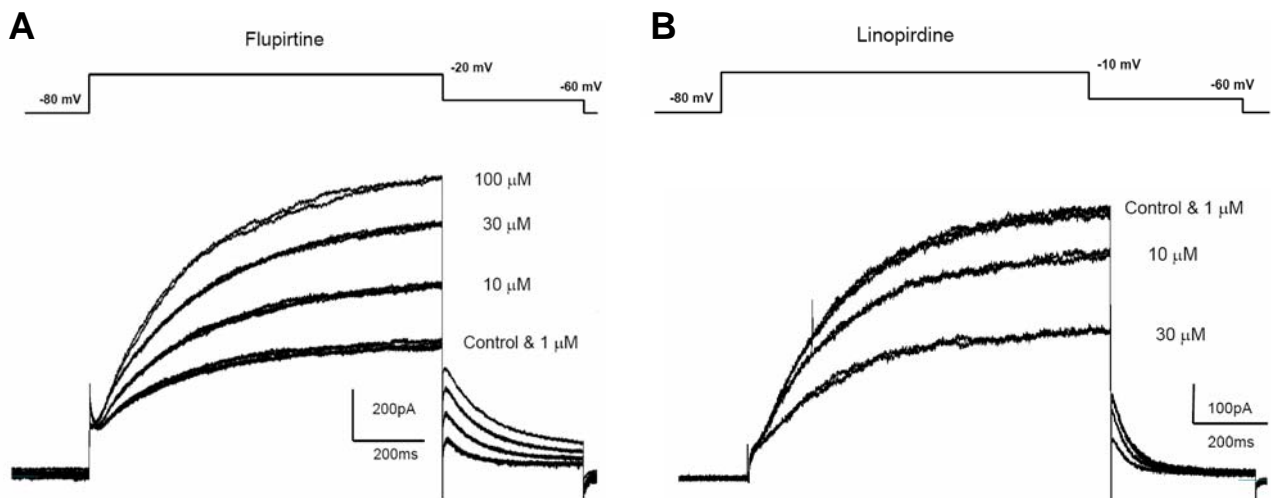


Figure 2. Flupirtine Potentiation and Linopirdine Block in Manual Patch Clamp

A: Superimposed current traces elicited by 1-s test pulses to -20 mV in the absence (control) and presence of an agonist, flupirtine. **B:** Superimposed current traces elicited by 1-s test pulses to -20 mV in the absence (control) and presence of an antagonist, linopirdine.

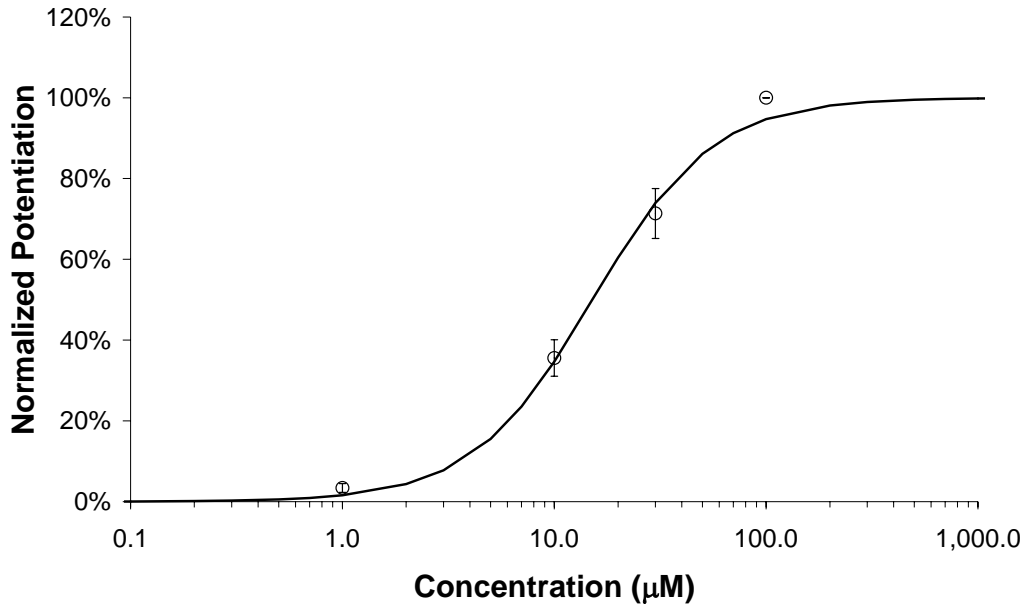


Figure 3. Flupirtine Concentration-Response Relationship

Percent potentiation normalized to the maximum level achieved in each cell (Mean \pm SEM, n = 3 cells/concentration). The maximum ranged from 93% to 129% of control at 100 μ M. EC₅₀ = 15.2 μ M.

2.2 PatchXpress[®]

2.2.1 Throughput Capability in PatchXpress[®]

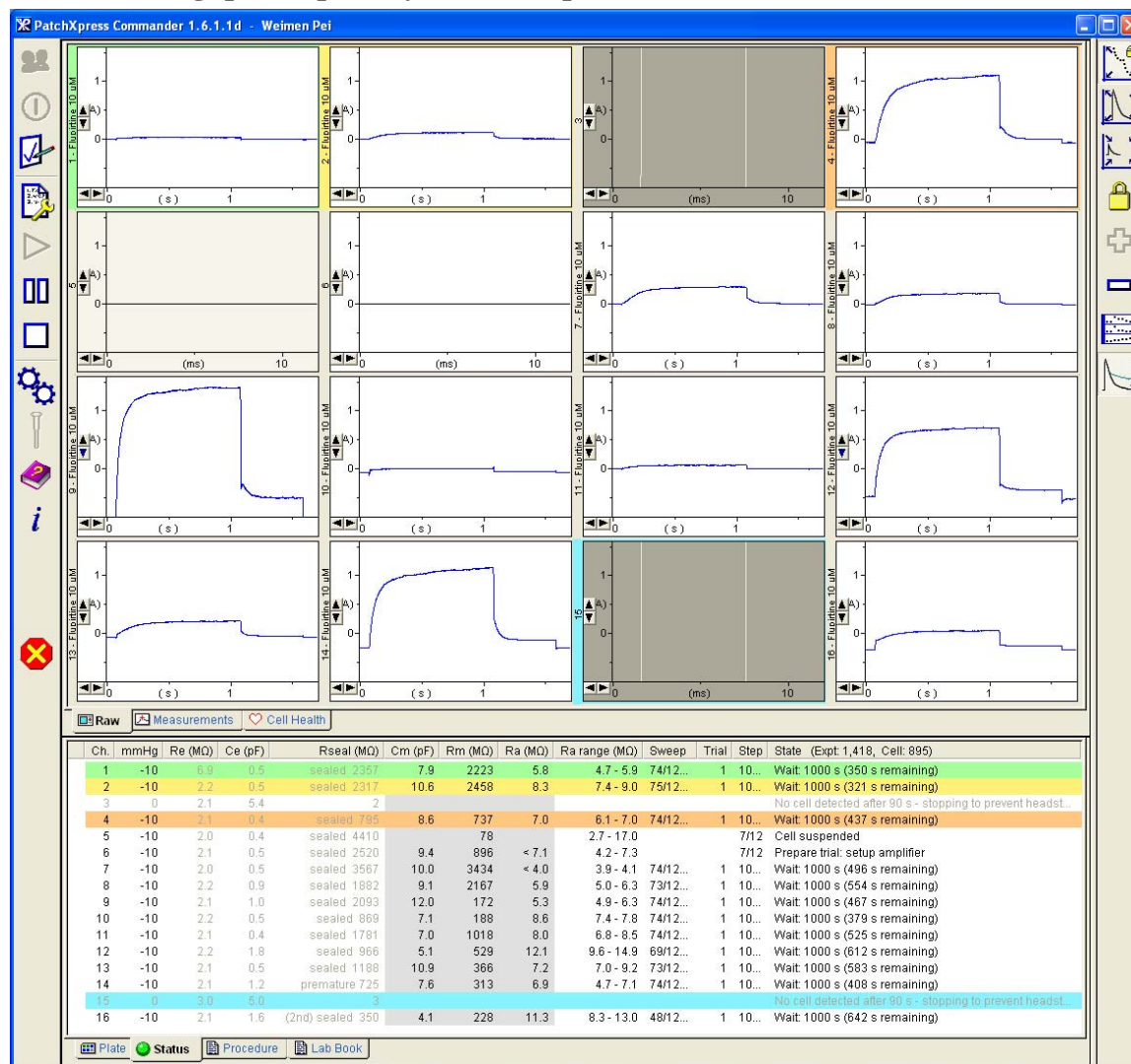


Figure 4. PatchXpress[®] Screen Capture.

Throughput capability in PatchXpress[®] depends upon many factors which may result in success rate variability. The screen capture shows a typical PatchXpress[®] experiment. In this example, 14 of a possible 16 seals were formed, whole-cell configuration was achieved in 9 cells, and all cells showed characteristic hK_v7.4 current waveforms with little leak current and peak current amplitudes > 0.2 nA.

2.3 QPatch™ HT

2.3.1 Throughput Capability in QPatch™ HT

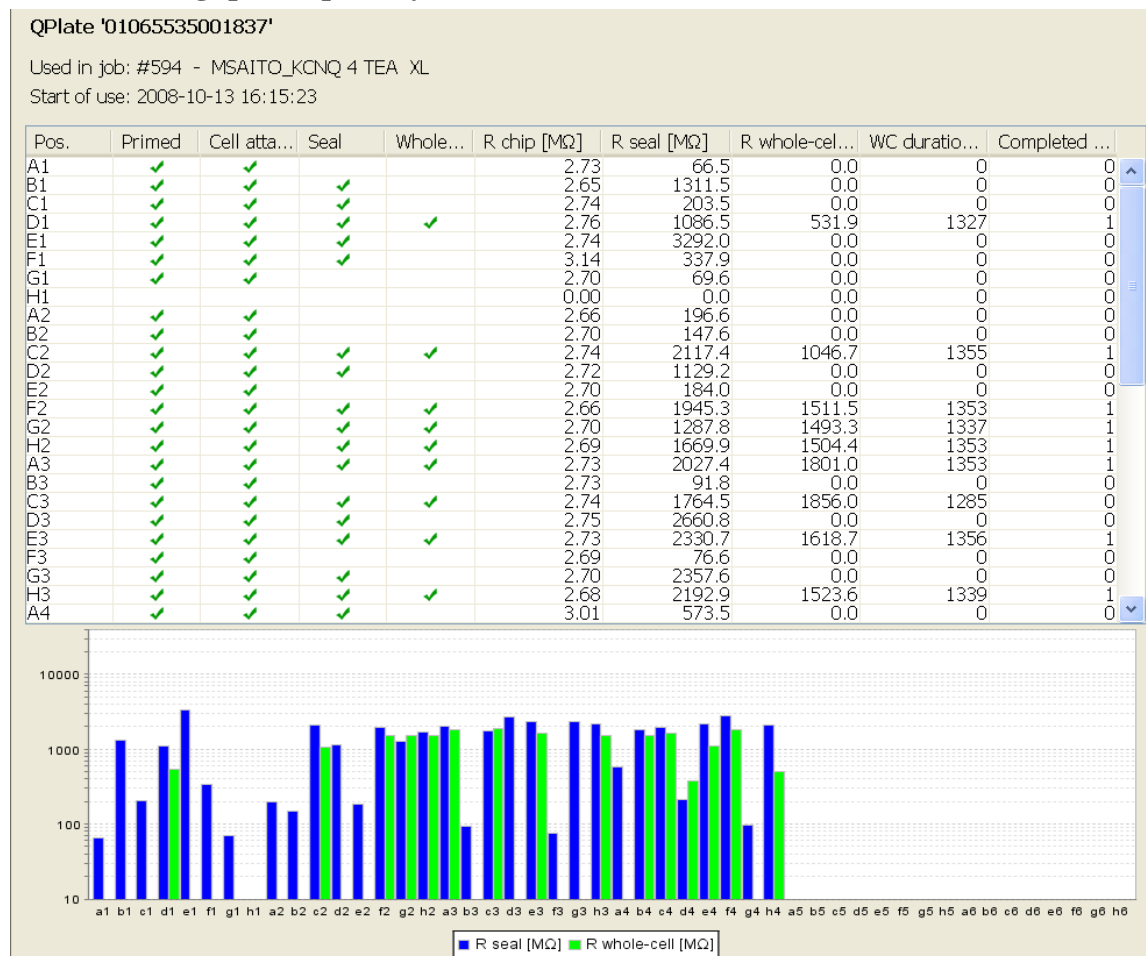


Figure 5. QPatch™ HT Screen Capture.

Throughput capability in QPatch™ HT depends upon many factors which may result in success rate variability. The screen capture shows a typical QPatch™ HT experiment. In this example, 29 of a possible 48 recording wells reached the whole cell recording configuration (whole cell success rate 60%). Twenty-eight recordings completed the experiment at 30 minutes.

2.3.2 Representative Data

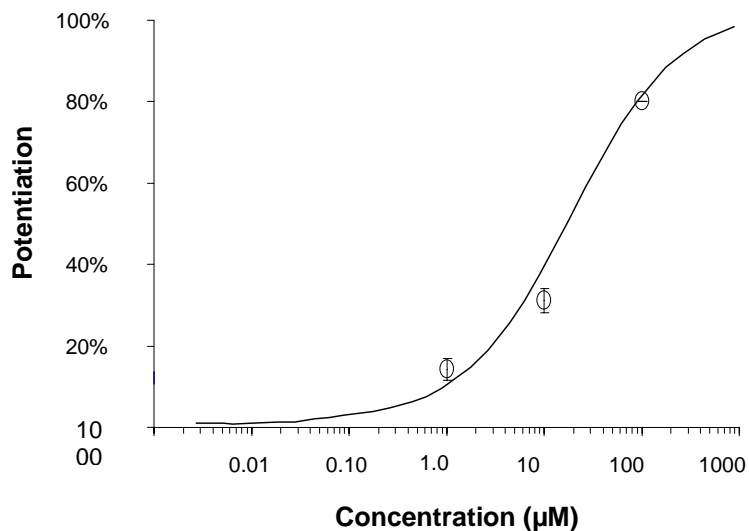


Figure 6. Agonist Effect of Flupirtine in QPatch™ HT
Flupirtine concentration-response relationship (Mean ± SEM, n = 4 cells/concentration, EC₅₀ = 21.5 µM).

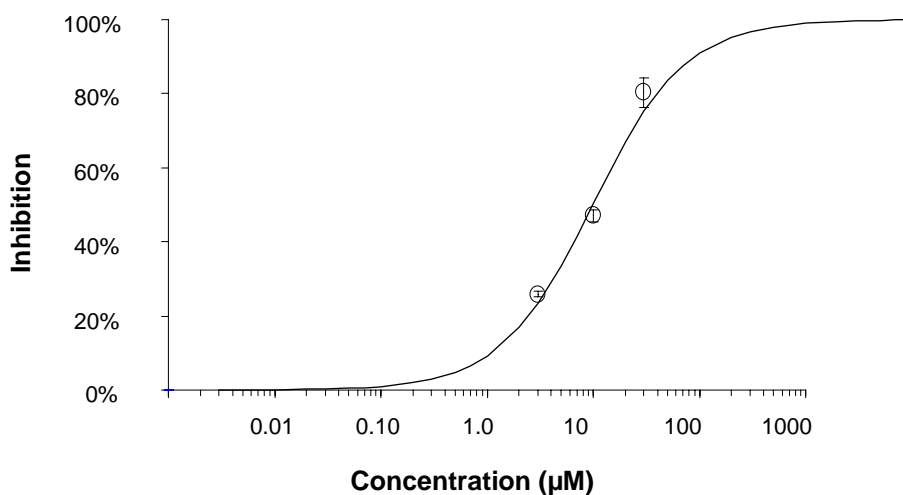


Figure 7. Antagonist Effect of Linopirdine in QPatch™ HT
Linopirdine concentration-response relationship (Mean ± SEM, n = 3 - 4 cells/concentration, EC₅₀ = 9.8 µM).

2.4 FLIPR Tetra[®] Representative Data

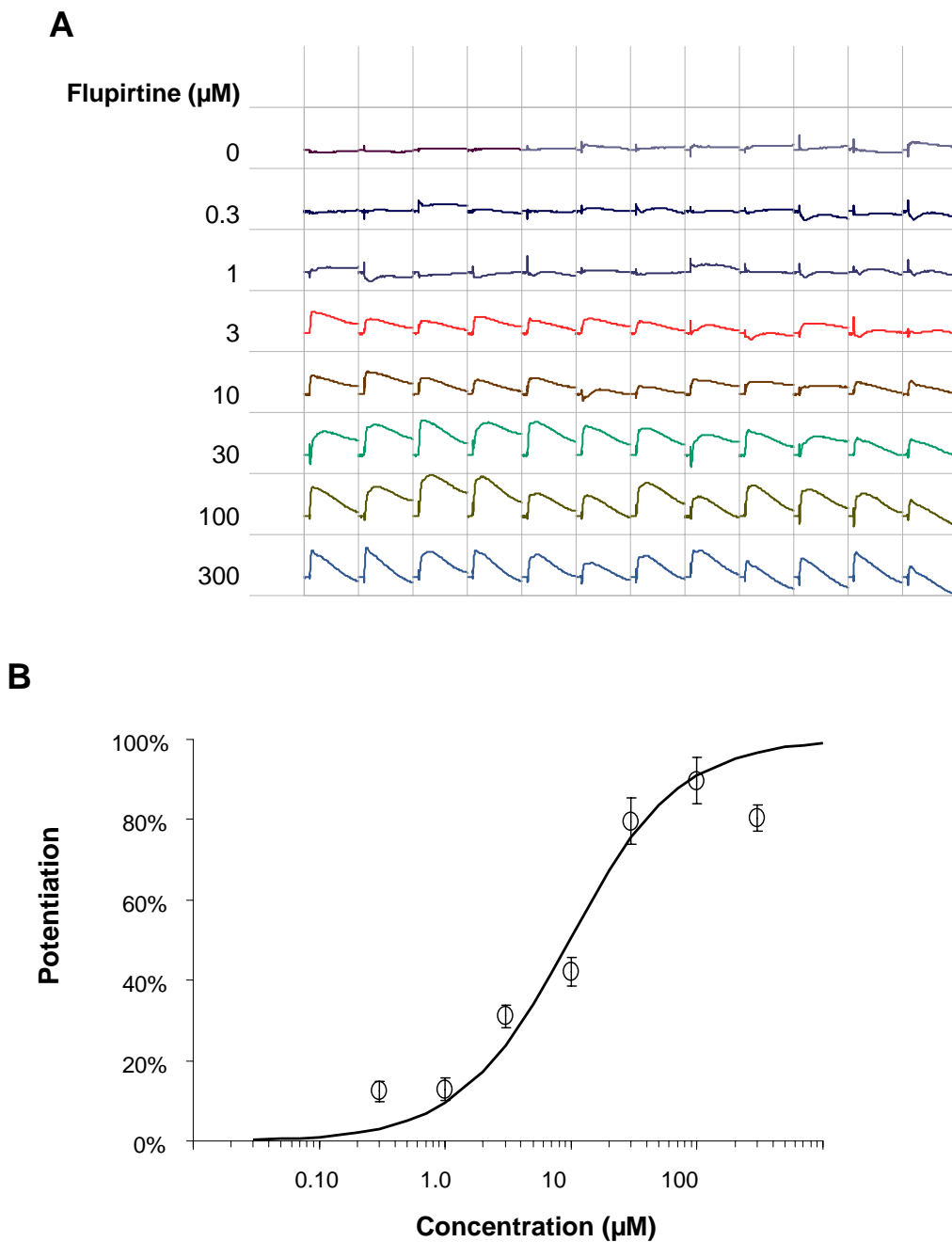


Figure 8. Activation by Flupirtine in FLIPR[®]

A: Flupirtine in K⁺-free buffer produced a concentration-dependent increase in TI⁺ flux in hK_v7.4-CHO cells. **B:** Flupirtine concentration-response relationship (Mean ± SEM, n = 12 replicates/concentration, EC₅₀ = 9.7 µM).

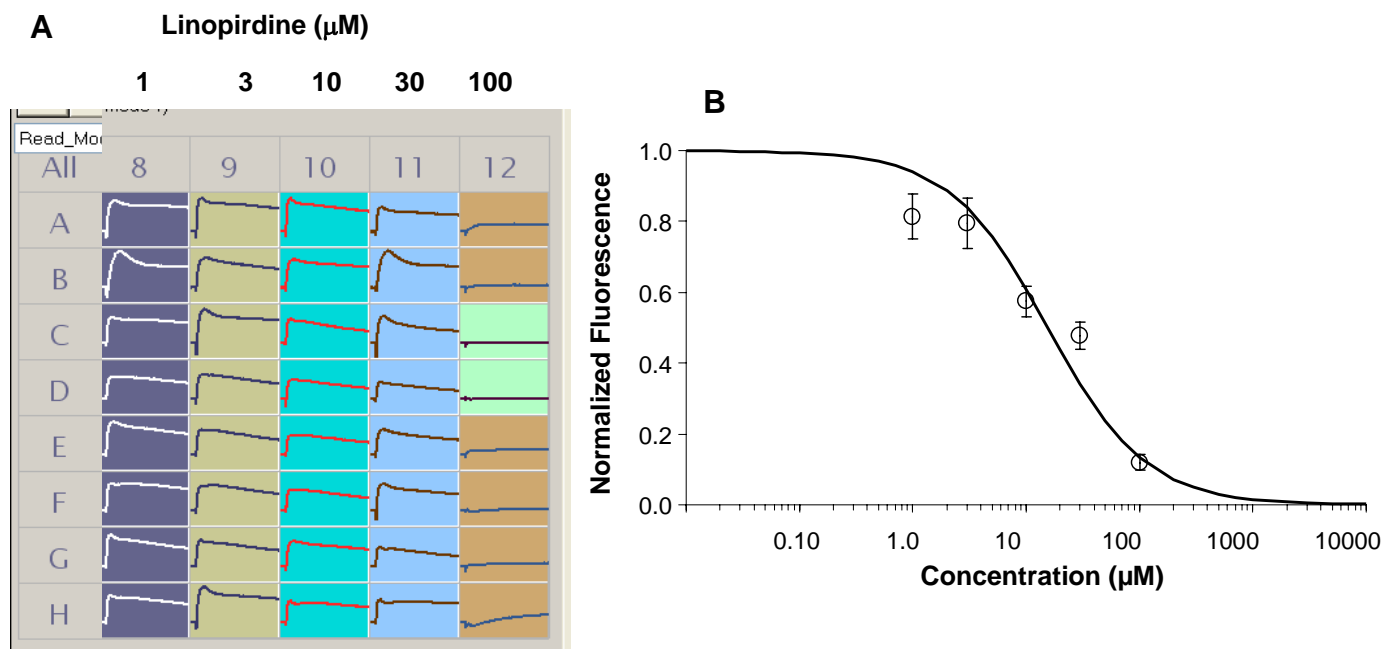


Figure 9. Linopirdine Inhibition of Flupirtine -Induced TI^+ Flux

A: Linopirdine inhibits flupirtine ($30 \mu\text{M}$)-induced TI^+ responses in hK_v7.4-CHO cells. **B:** Concentration-response relationship ($\text{IC}_{50} = 15.6 \mu\text{M}$).

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.