



Stably Transfected Cell Line - Product Data Sheet
hK_{Ca}2.2-CHO
Catalog Number CT6181

Related Services and Products

FastPatch[®] and ScreenPatch[™] automated patch clamp services
Additional information available at www.chantest.com

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1 Cell Line Description

1.1 Background

K_{Ca}2.2 is a small-conductance (2 - 3 pS single channel conductance), potassium-selective, channel that is activated by increased intracellular [Ca²⁺]. Expressed in neurons, hK_{Ca}2.2 channels contribute to afterhyperpolarization that modulates repetitive firing frequency.

1.2 Pore-forming subunit identifier: hK_{Ca}2.2

Synonym: SK2

Class: Calcium-activated potassium channel

Species: Human

Gene name: KCNN2

1.3 Sequence Information

The cDNA sequence of the KCNN2 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_021614.2

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.).

1.7 Cell Line Stability

Channel expression in this cell line has been for at least 56 passages.

2 Validated Test Platforms

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

QPatch™ HT (Sophion)

FLIPR® (MDS-AT)

2.1 QPatch™ HT Representative Data

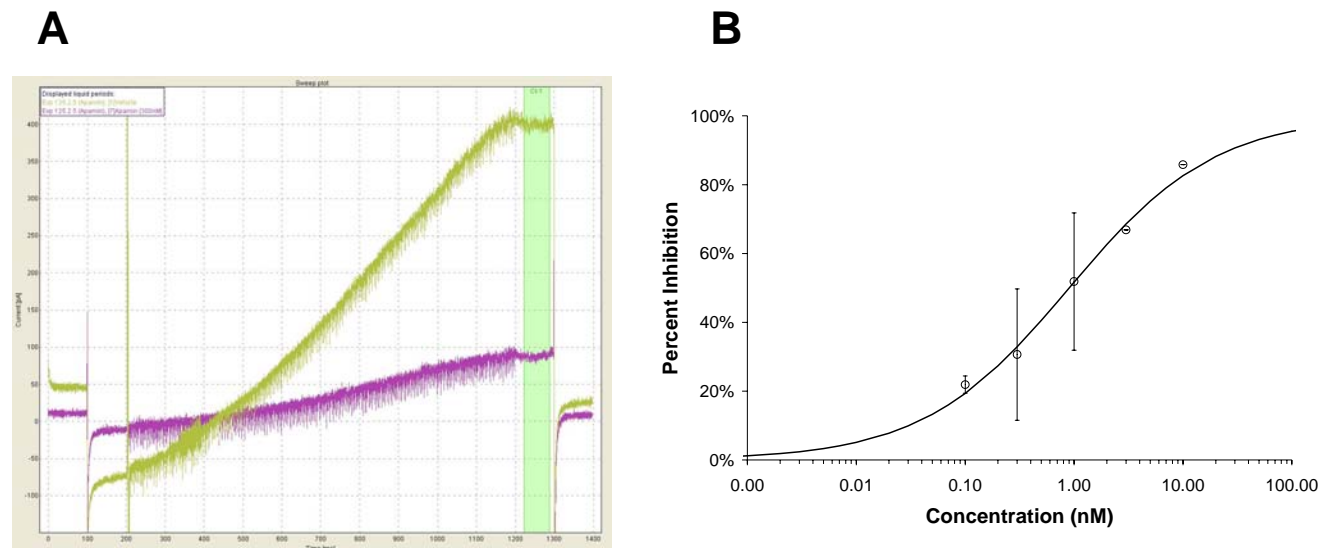


Figure 1. Apamin block of hK_{Ca}2.2 in QPatch™ HT.

A: Test pulse currents elicited by voltage ramps from -110 to +40 mV, before and after applying a saturating concentration (300 nM) of the SK channel antagonist, apamin. Horizontal and vertical calibrations, 100 ms and 50 pA, respectively. **B:** Concentration-response relationship (Mean ± SEM, n = 1 - 2), IC₅₀ = 0.9 nM.

2.2 FLIPR Tetra[®] Representative Data

2.2.1 Apamin Inhibition of A23187-activated hK_{Ca}2.2 Channels

Channels were activated with a saturating concentration of the calcium ionophore A23187 (30 μ M). Apamin, a selective antagonist, inhibited TI⁺ influx in a concentration-dependent manner.

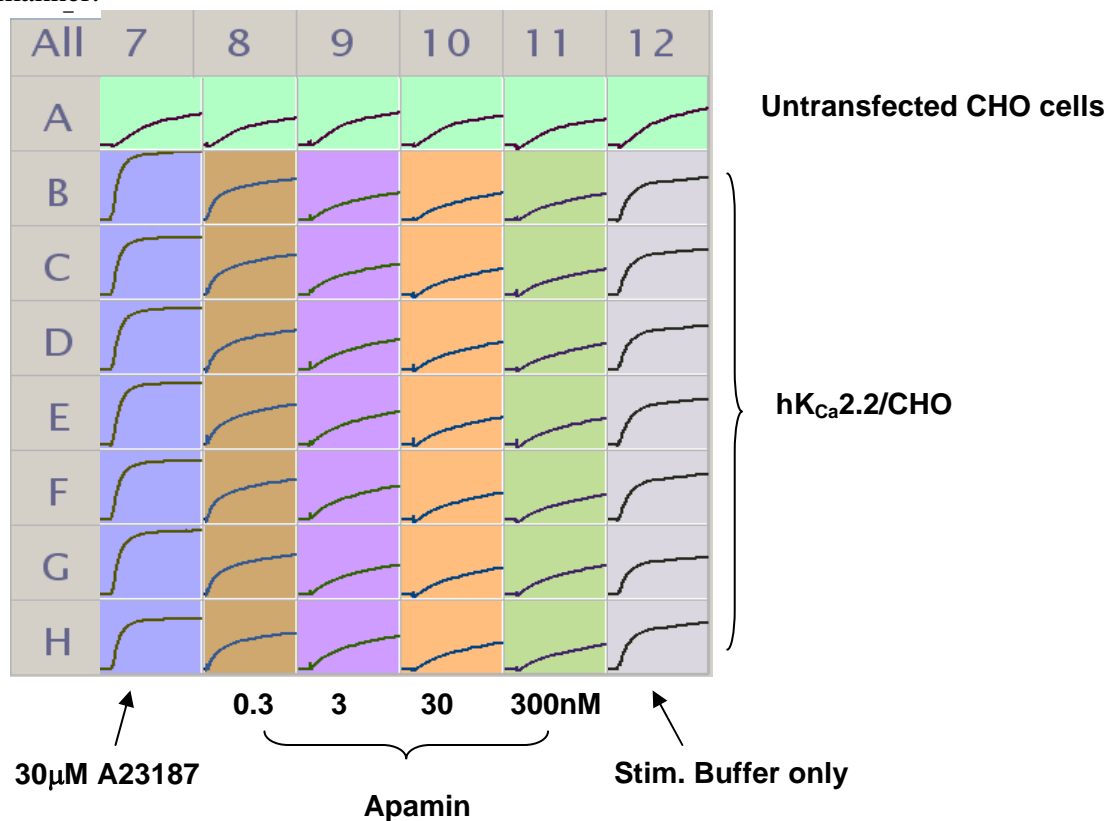


Figure 2. Apamin inhibition of TI⁺ influx.

A saturating concentration (30 μ M) of A23187 activated TI⁺ influx via hK_{Ca}2.2 channels. The TI⁺ signal was suppressed by increasing concentrations of apamin. The signal elicited in stimulus buffer without A23187 shows the baseline response. Signals in the presence of 300 nM apamin are smaller than those in stimulus buffer alone suggesting that some apamin-sensitive channels were active before A23187 addition.

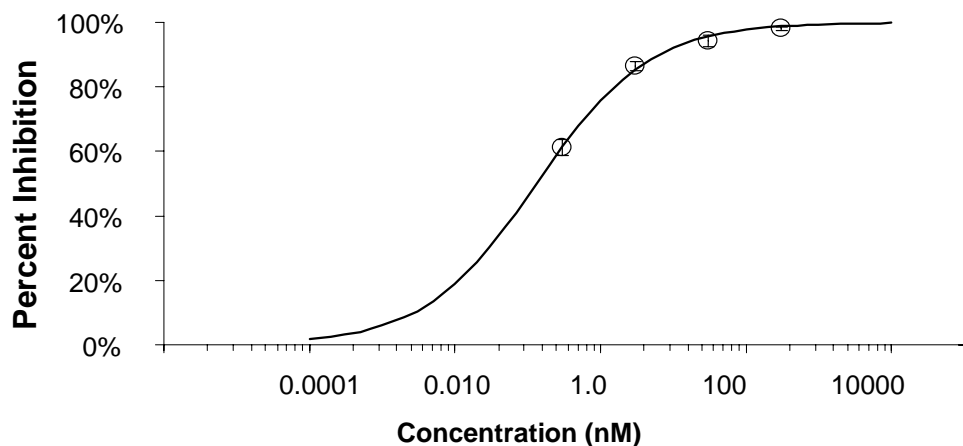


Figure 3. Apamin Concentration-Response Relationship
Mean \pm SEM, n = 7 replicates/concentration. IC₅₀ = 0.13 nM.

2.2.2 UCL1684 Inhibition of A23187-activated hK_{Ca}2.2 channels.

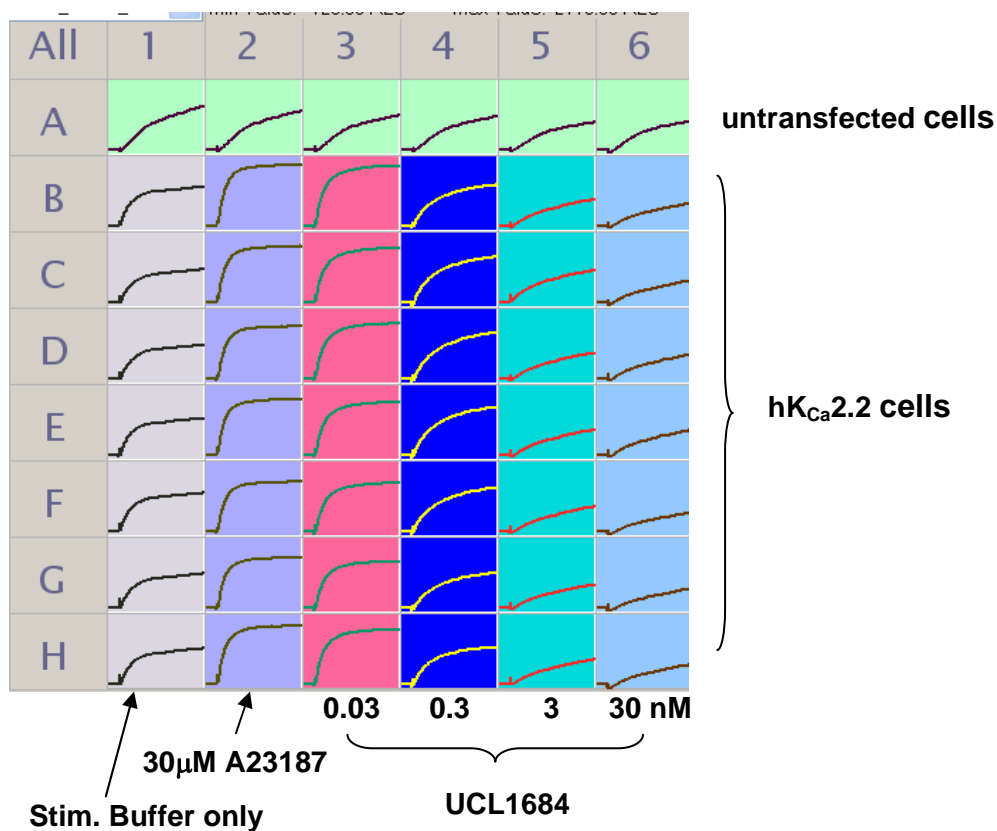


Figure 4. UCL1684 inhibition of TI⁺ influx.

A saturating concentration (30 μ M) of A23187 activated hK_{Ca}2.2 channels. TI⁺ influx was completely inhibited by 30 nM UCL1684 and indistinguishable from the signal elicited in untransfected cells. The wells marked 'Stim Buffer only' (left-hand column) show the signals elicited by stimulus buffer without A23187. The signal in the presence

of 30 nM UCL1684 is smaller than that of stimulus buffer alone suggesting that some UCL1684-sensitive channels were active before A23187 addition

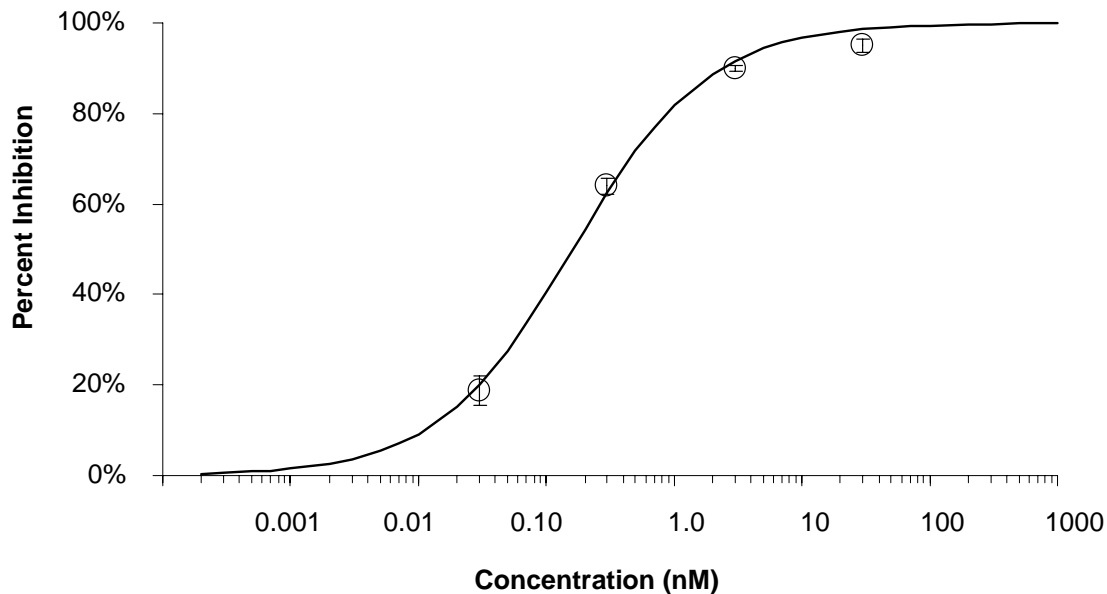


Figure 5. UCL1684 Concentration-Response Relationship

Mean \pm SEM, n = 7 replicates/concentration. IC₅₀ = 0.2 nM.

3 References

Fanger CM, et al. 2001. Calcium-activated potassium channels sustain calcium signaling in T lymphocytes. *J Biol Chem* 276: 12249–12256.

Strøbæk D, et al. 2000. Pharmacological characterization of small-conductance Ca²⁺-activated K⁺ channels stably expressed in HEK 293 cells. *Br J Pharmacol.* 129: 991–999.

Wei AD, et al. International Union of Pharmacology. LII. 2005. Nomenclature and molecular relationships of calcium-activated potassium channels. *Pharmacol Rev.* 57:463-472.