



Stably Transfected Cell Line - Product Data Sheet
hK_{Ca}2.3-CHO
Catalog Number CT6182

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}2.3 is a small-conductance, potassium-selective, voltage-insensitive channel that is activated by increased intracellular [Ca²⁺]. hK_{Ca}2.2 is widely distributed in neuronal, endothelial, and muscle cells. Expressed in neurons, K_{Ca}2.3 channels contribute to afterhyperpolarization that modulates repetitive firing frequency. Activators have therapeutic potential in treatment of epilepsy, hypertension, and urinary incontinence.

1.2 Pore-forming subunit identifier: hK_{Ca}2.3

Synonym: SK3

Class: Calcium-activated potassium channel

Species: Human

Gene name: KCNN3

1.3 Sequence Information

The cDNA sequence of the KCNN3 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_002249.4

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 stable 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:

Manual Patch Clamp

PatchXpress[®]

QPatch[™] HT (Sophion)

FLIPR[®] (MDS-AT)

2.1 Manual Patch Clamp Representative Data

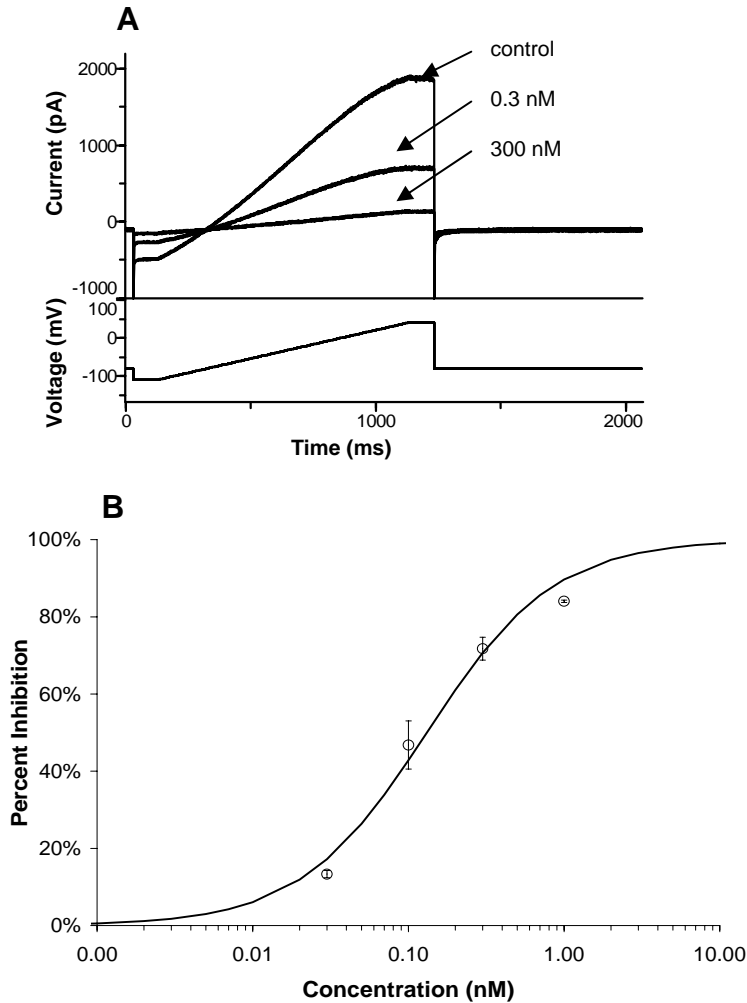


Figure 1. Apamin Block of hK_{Ca}2.3 in Manual Patch Clamp

A: Current traces elicited by ramp depolarizations -110 to +40 mV in before (control) and after application of apamin, a selective blocker. **B:** Concentration-response relationship. Mean \pm SEM (n = 3 - 4 cells/concentration). IC₅₀ = 0.13 nM.

2.2 PatchXpress[®] Representative Data

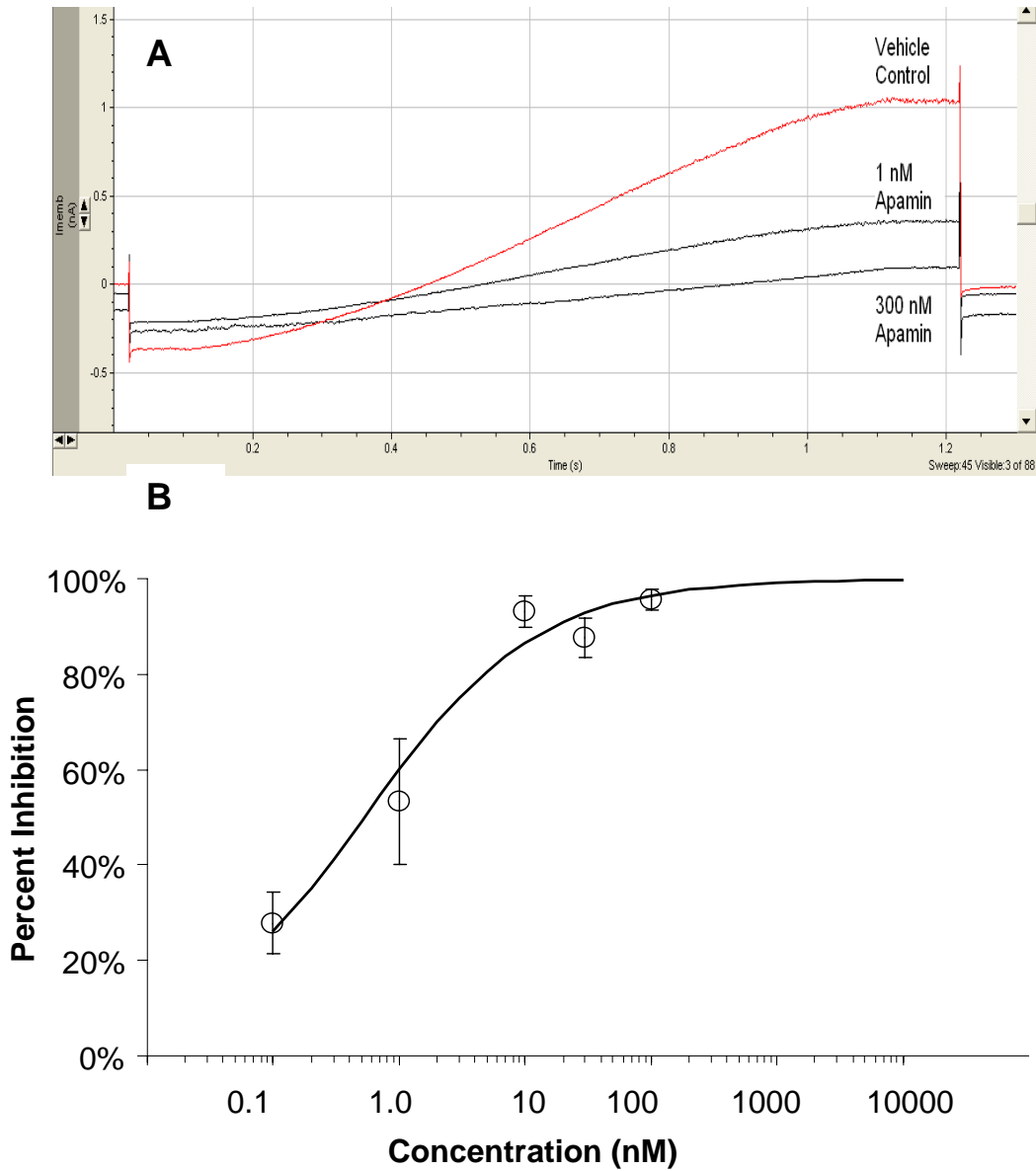


Figure 2. Apamin Block in PatchXpress[®]

A: Current traces elicited by ramp depolarizations -110 to +40 mV in before (control) and after application of apamin, a selective blocker. **B:** Concentration-response relationship. Mean \pm SEM (n = 3 - 6 cells/concentration). IC₅₀ = 1 nM.

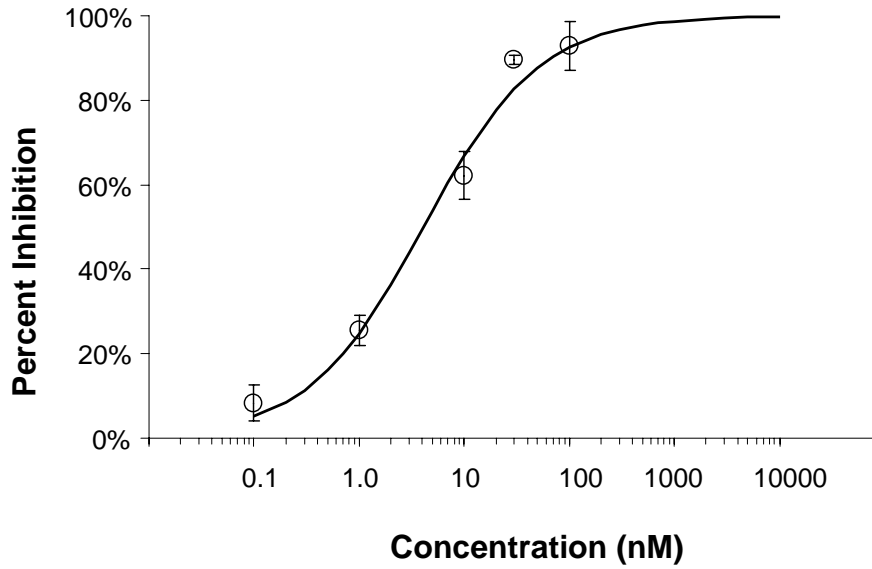


Figure 3. UCL 1684 Concentration-Response Relationship
Mean ± SEM (n = 3 - 5 cells/concentration). IC₅₀ = 4 nM.

2.3 QPatch™ HT Representative Data

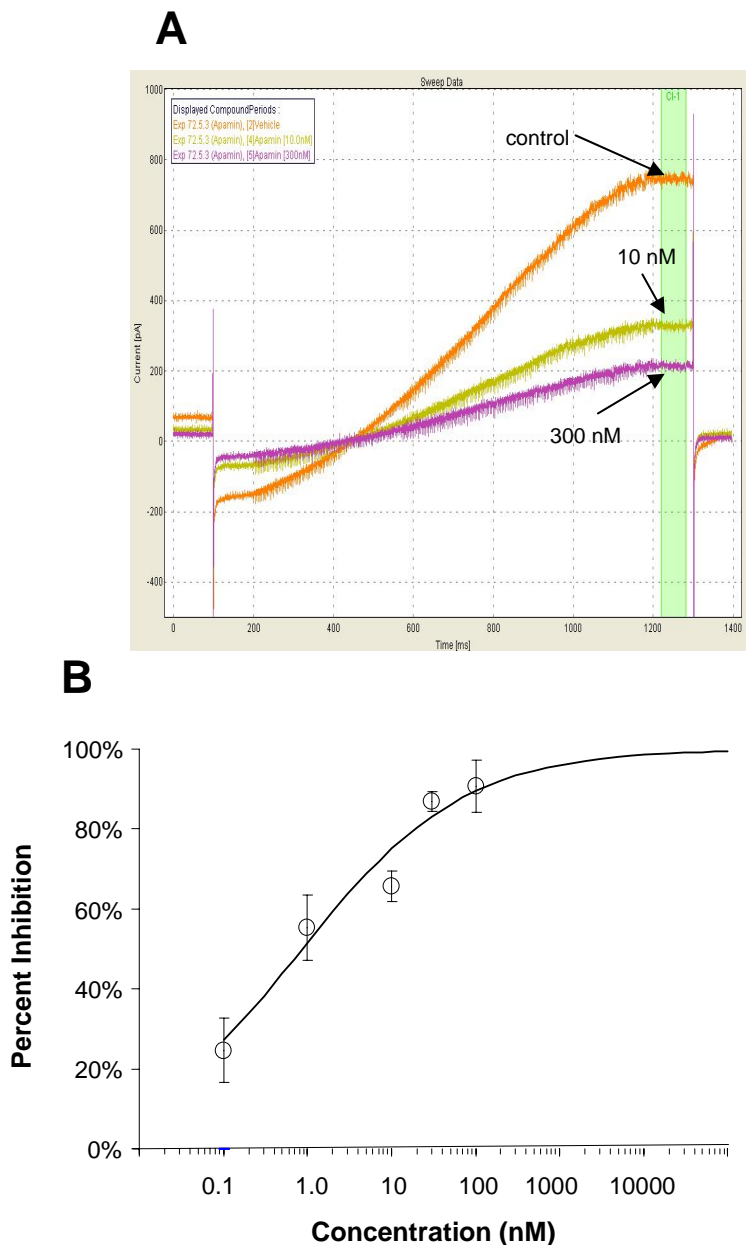


Figure 4. Apamin block of hK_{Ca}2.3 in QPatch™ HT.

A: Current traces elicited by ramp depolarization (-110 to +40 mV) before (control) and after 10 and 300 nM apamin application. Horizontal and vertical calibrations, 200 ms and 0.2 pA, respectively. **B:** Concentration-response relationship (Mean ± SEM, n = 5 - 9 cells/concentration). IC₅₀ = 1 nM.

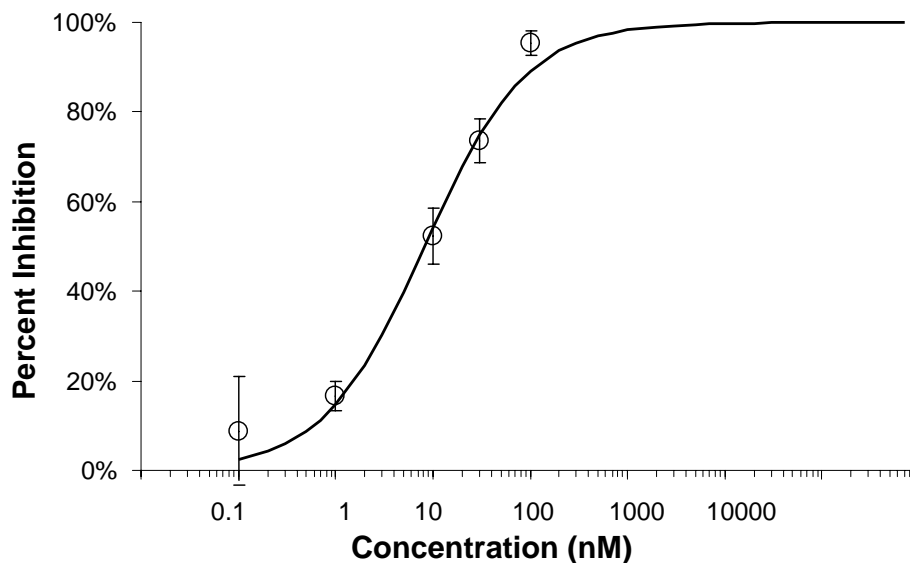


Figure 5. UCL 1684 Concentration-Response Relationship
Mean \pm SEM (n = 2 - 4 cells/concentration). IC₅₀ = 8 nM.

2.4 Representative FLIPR Tetra[®] Data

2.4.1 Apamin Inhibition of A23187-activated hK_{Ca}2.3 Channels

Channels were activated with a saturating concentration of the calcium ionophore A23187 (10 μ M). Apamin, a selective antagonist, inhibited TI⁺ influx in a concentration-dependent manner.

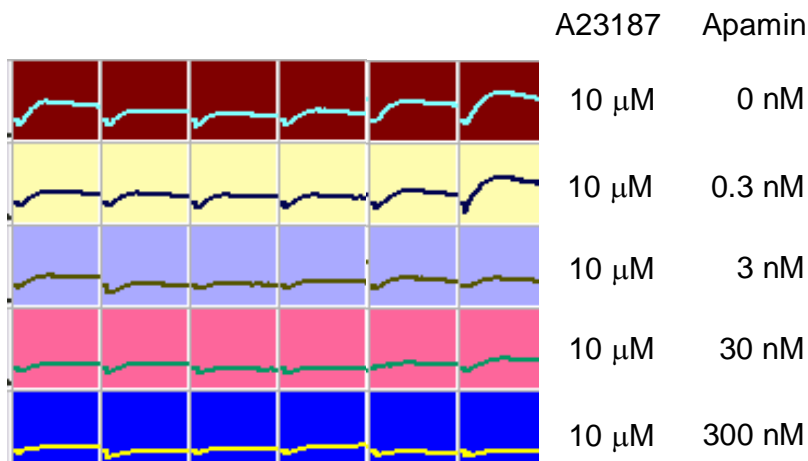


Figure 6. Apamin inhibition of TI⁺ influx.

A23187 at 10 μ M activated TI⁺ influx via hK_{Ca}2.3 channels. The TI⁺ signal was suppressed by increasing concentrations of apamin (0.3 and 100 nM).

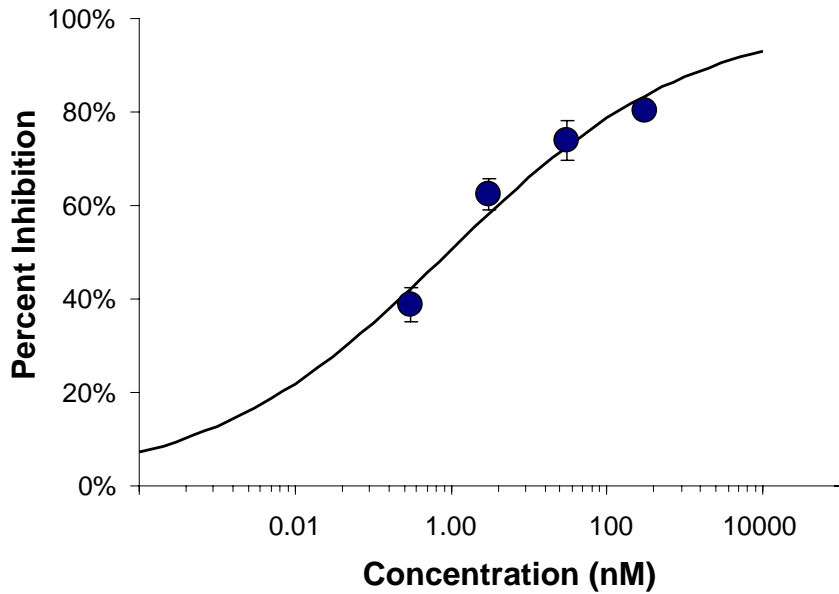


Figure 7. Apamin Concentration-Response Relationship
Mean \pm SEM, n = 6 - 8 replicates/concentration. IC₅₀ = 0.9 nM.

2.4.2 UCL1684 Inhibition of A23187-activated hK_{Ca}2.3 channels.

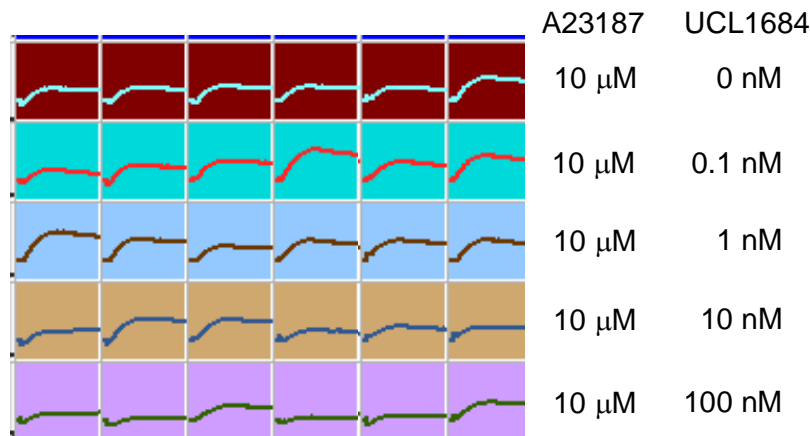


Figure 8. UCL1684 inhibition of TI⁺ influx.
A23187 at 10 μ M activated hK_{Ca}2.3 channels. TI⁺ influx was inhibited in a concentration-dependent manner by increasing UCL1684.

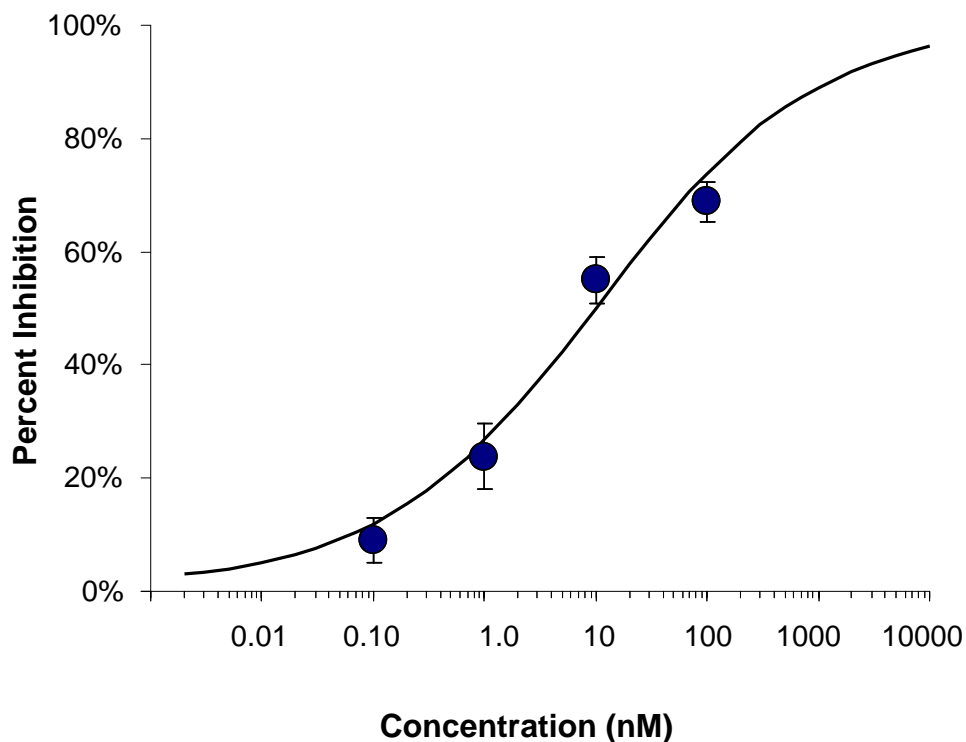


Figure 9. UCL1684 Concentration-Response Relationship
Mean ± SEM, n = 6 replicates/concentration. IC₅₀ = 10.7 nM.

3 References

Barford ET, et al. 2001. Cloning and functional expression of a liver isoform of the small conductance Ca²⁺-activated K⁺ channel SK3. *Am J Physiol Cell Physiol* 280:C836–C842

Hosseini R, et al. 2001. SK3 is an important component of K⁺ channels mediating the afterhyperpolarization in cultured rat SCG neurons. *J Physiol* 535 2:323–334

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