



hK_{ir}6.2/SUR2A-HEK Product Data Sheet
Stably Transfected Cell Line
Catalog Number CT6183

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_{ir}6.2/SUR2A is an inwardly-rectifying, ATP-sensitive, K⁺-selective channel. In the heart ischemia-induced channel activation hyperpolarizes the myocardial cells.

1.2 Pore-forming subunit identifier: hK_{ir}6.2

Class: Inwardly rectifying potassium channel
Species: Human
Gene name: KCNJ2

1.3 Auxiliary subunit identifier: SUR2A

Class: ATP-binding cassette
Species: Human
Gene name: ABCC9

1.4 Sequence Information

The cDNA sequences of the genes used to create this cell line were confirmed prior to transfection. The amino acid sequences encoded by the transfected KCNJ2 and ABCC9 cDNAs are identical to the translated sequence for GenBank accession number NM_000525.3 and NM_005691.2, respectively.

1.5 Expression System

HEK293 (human embryonic kidney) cells, inducible expression.

1.6 Product Format

Cryopreserved cells, 1 x10⁶ cells/vial.

1.7 Mycoplasma Status: Negative

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

1.8 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:

PatchXpress® (MDS-AT)
FLIPR® (MDS-AT)

3 Validation Results

3.1 PatchXpress[®]

3.1.1 Throughput Capability in PatchXpress[®]

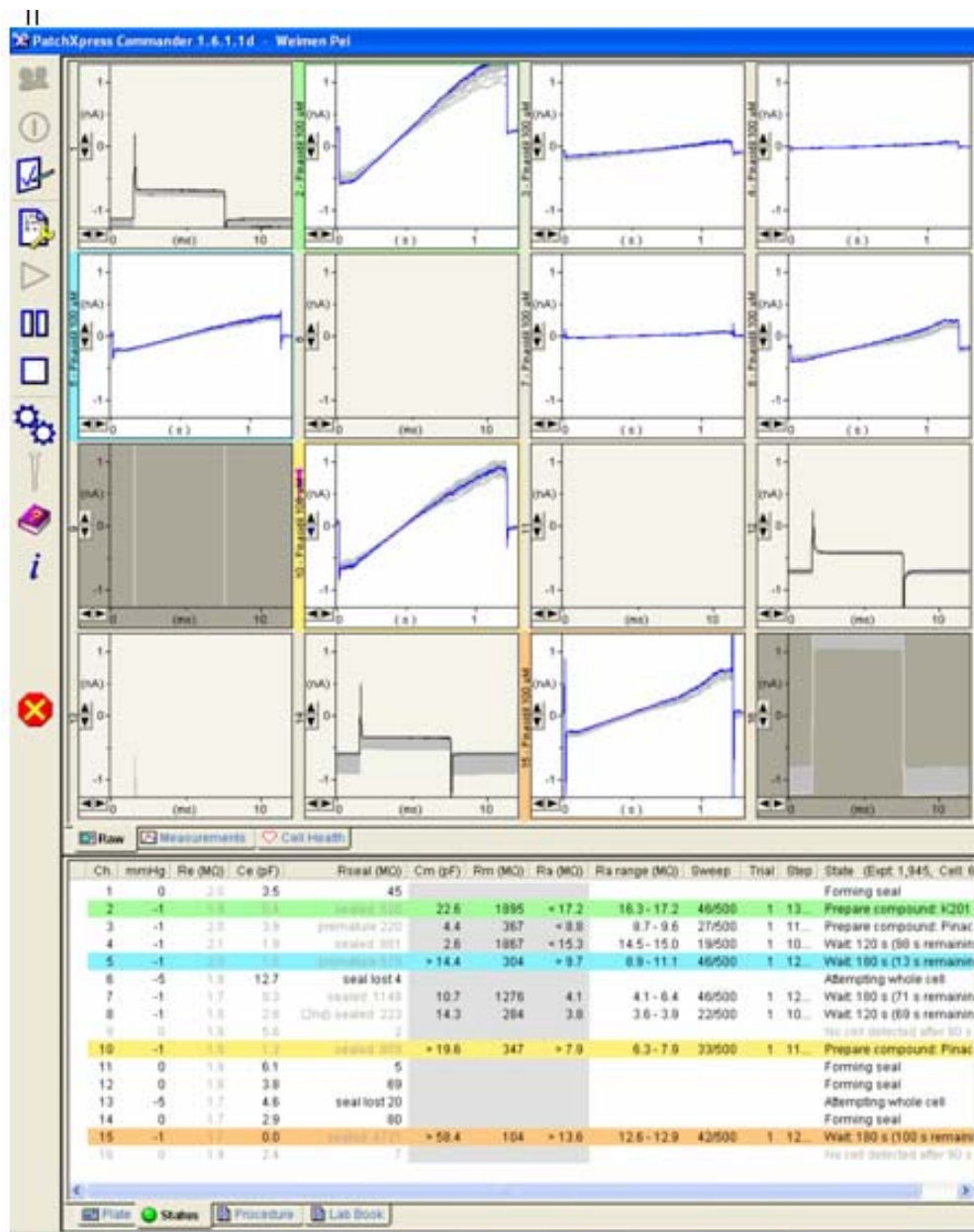


Figure 1. PatchXpress[®] hK_{ir}6.2/SUR2A-HEK Screen Capture.

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, whole-cell configuration was achieved in 8 cells, and 5 cells showed characteristic hK_{ir}6.2/SUR2A current waveforms.

3.1.2 Validation Results

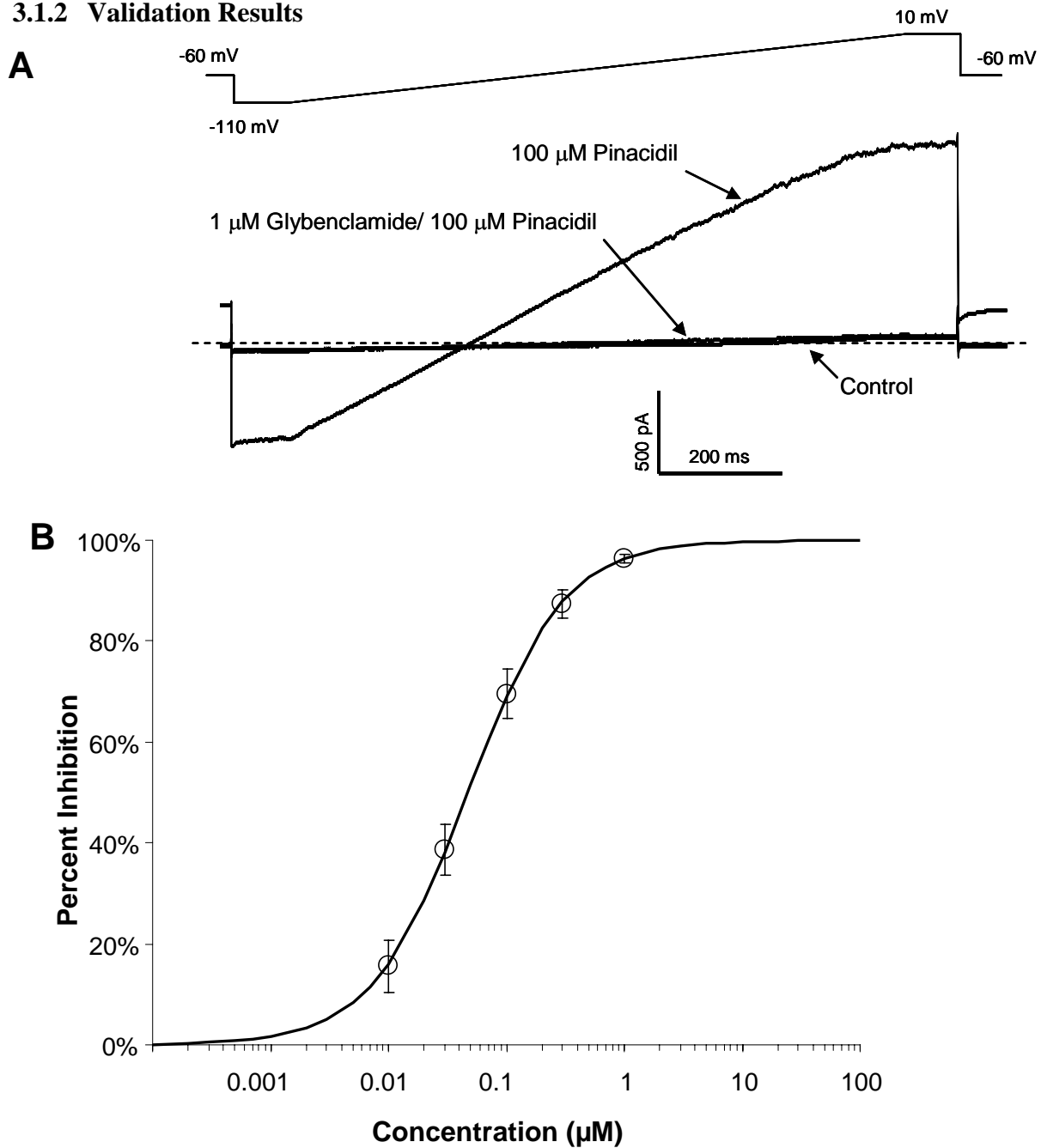


Figure 2. Pharmacological Characteristics of hK_{ir}6.2/SUR2A-HEK in PatchXpress[®]

A: Pinacidil-induced activation and glibenclamide inhibition of hKir6.2/SUR2A channels. Currents elicited by voltage ramps (upper panel) from -110 to +10 mV, holding potential -60 mV. **B:** Concentration-response relationship for glibenclamide inhibition. Mean \pm SEM, n = 2 - 3 cells/concentration, IC₅₀ = 0.05 μ M.

3.2 Representative FLIPR® Data

3.2.1 Activation of Kir6.2/SUR2A by pinacidil

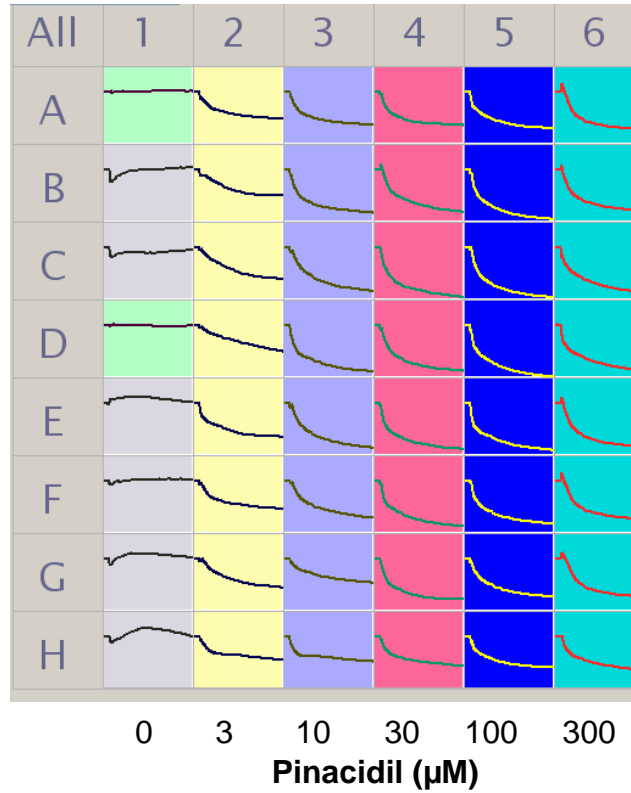


Figure 3. Concentration-Dependent Activation of Kir6.2/SUR2A channels in FLIPR®

Activation of K_{ir}6.2/SUR2A channels hyperpolarizes the membrane potential and is detected as a downward signal deflection. Increasing concentrations of pinacidil produced concentration-dependent hyperpolarization.

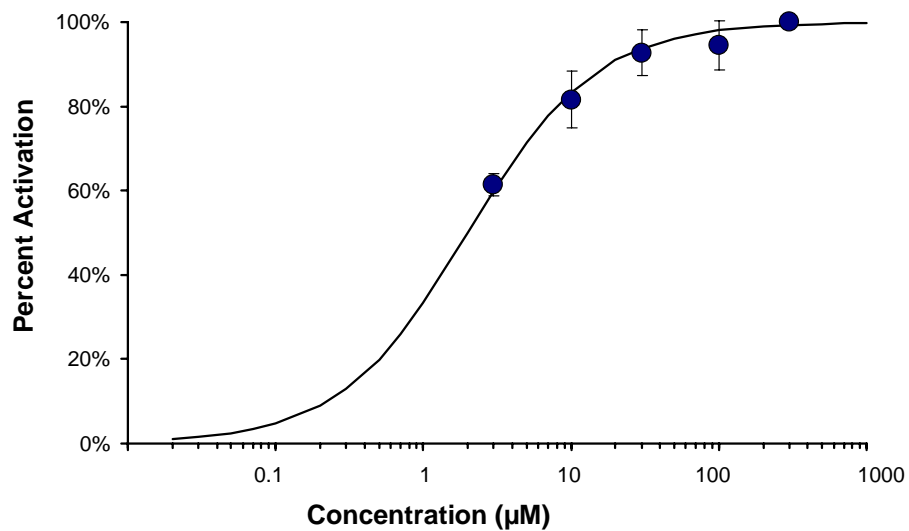


Figure 4. Concentration-response curve of Kir6.2/SUR2A by pinacidil.
Mean \pm SEM, n = 7 - 8 replicates/concentration. EC₅₀ = 2.0 μ M.

3.2.2 Inhibition of hKir6.2/SUR2A by glibenclamide

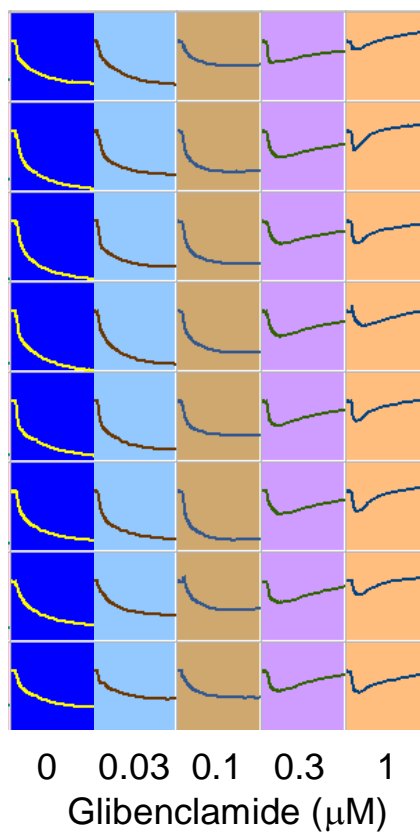


Figure 5. Glibenclamide inhibits hKir6.2/SUR2A channels activated by 100 μM pinacidil. Pinacidil (100 μM)-induced activation was inhibited by glibenclamide in a concentration-dependent manner (0.03 - 1 μM).

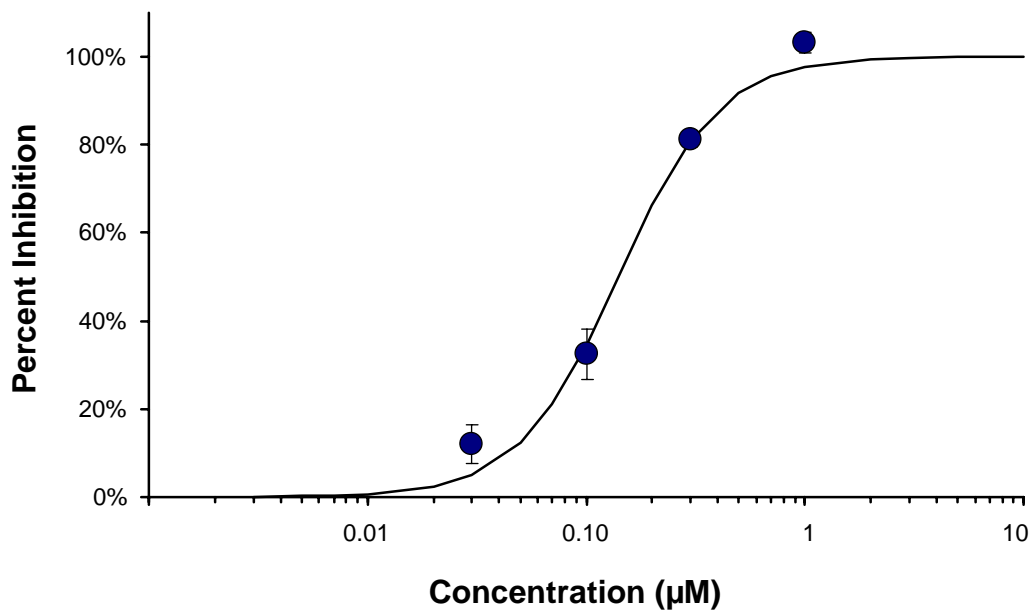


Figure 6. Glibenclamide Concentration-Response Relationship

Mean \pm SEM, n = 6 - 8 replicates/concentration. IC₅₀ = 0.14 μ M.

4 References

Kubo Y, et al., International Union of Pharmacology. LIV. Nomenclature and molecular relationships of inwardly rectifying potassium channels. *Pharmacol Rev.* 2005 57:509-526.

Shindo T, et al. 1998. SUR2 subtype (A and B)-dependent differential activation of the cloned ATP-sensitive K⁺ channels by pinacidil and nicorandil. *Br J Pharmacol* 124:985-991.