



**hK<sub>ir</sub>3.1/K<sub>ir</sub>3.4-HEK Data Sheet**  
**Stably Transfected Cell Line**  
**Catalog Number CT6017**

**Related Services and Products**

FastPatch<sup>®</sup> and ScreenPatch<sup>™</sup> automated patch clamp services  
Additional information available at [www.chantest.com](http://www.chantest.com)

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## 1 Cell Line Description

### 1.1 Background

K<sub>ir</sub>3.1/K<sub>ir</sub>3.4 is an inwardly-rectifying, K<sup>+</sup>-selective channel that is activated by G-protein coupled receptors. In the heart, coupling with muscarinic M2 receptors generates the acetylcholine-induced potassium current, I<sub>KACH</sub>, responsible for vagal inhibition.

### 1.2 Pore-forming subunit identifier: hK<sub>ir</sub>3.1

Class: Inwardly rectifying potassium channel

Species: Human

Gene name: KCNJ3

### 1.3 Pore-forming subunit identifier: hK<sub>ir</sub>3.4

Class: Inwardly rectifying potassium channel

Species: Human

Gene name: KCNJ5

### 1.4 Sequence Information

The cDNA sequences of the KCNJ3 and KCNJ5 genes used to create this cell line were confirmed prior to transfection. The amino acid sequences encoded by the transfected cDNAs are identical to the translated sequence for GenBank accession numbers NM\_002239.2 and NM\_000890.3, respectively.

### 1.5 Expression System

HEK293 (human embryonic kidney) cells, constitutive expression.

### 1.6 Product Format

Cryopreserved cells, 1 x10<sup>6</sup> 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

**Table 1. Stability of hKir3.1/hKir3.4 Current**

Passage Number	Current Amplitude (pA)	n
15	1038 ± 489	6
19	539 ± 99	9
31	1305 ± 330	15

hKir3.1/hKir3.4 currents recorded by PatchXpress<sup>®</sup> using hKir3.1/hKir3.4-HEK (Mean ± SEM). Current amplitudes remain stable for at least 16 passages beyond the original passage (P15).

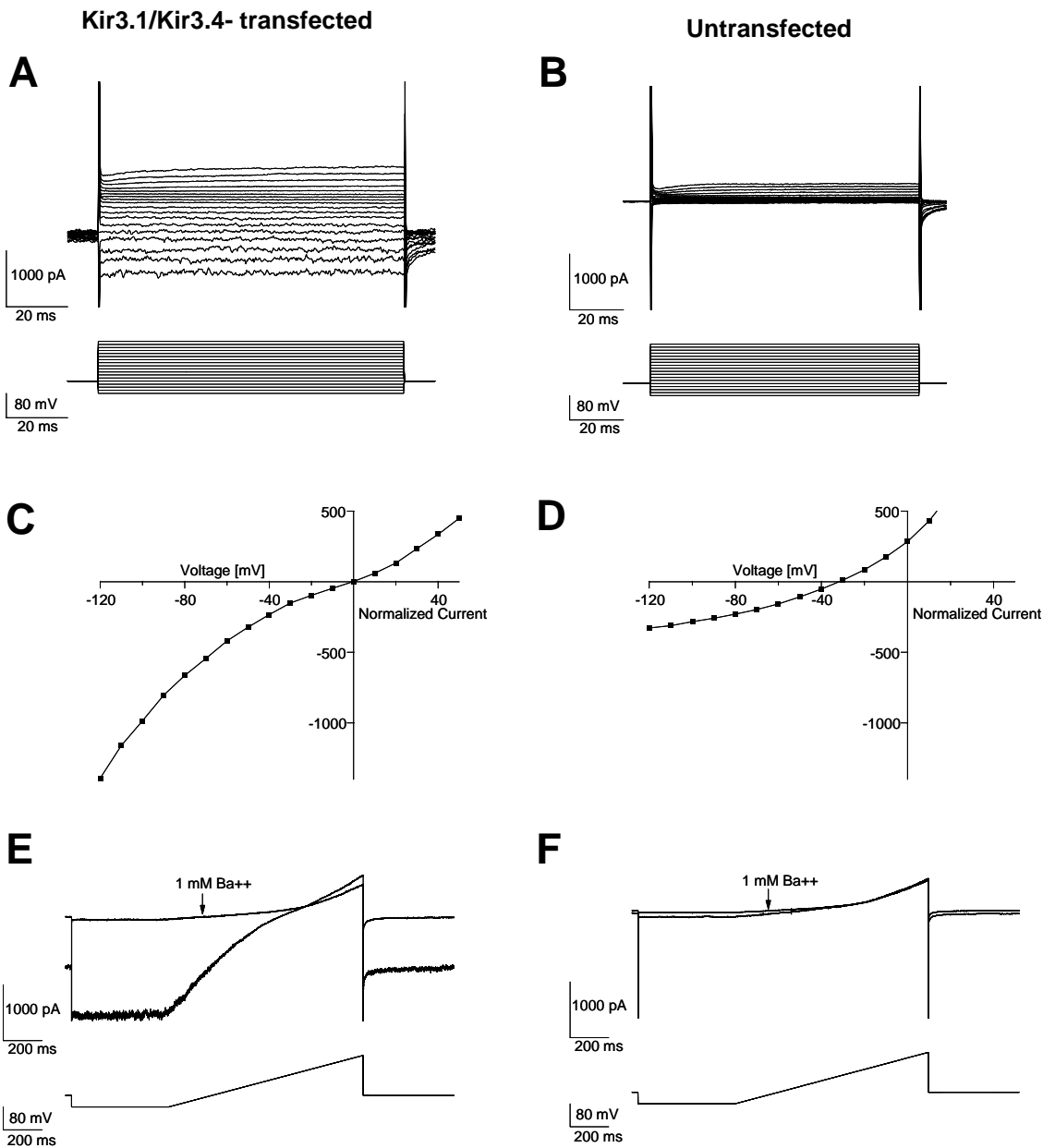
## 2 Validated Test Platforms

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

Manual Patch Clamp

PatchXpress<sup>®</sup> (MDS-AT)

## 2.1 Manual Patch Clamp Representative Data

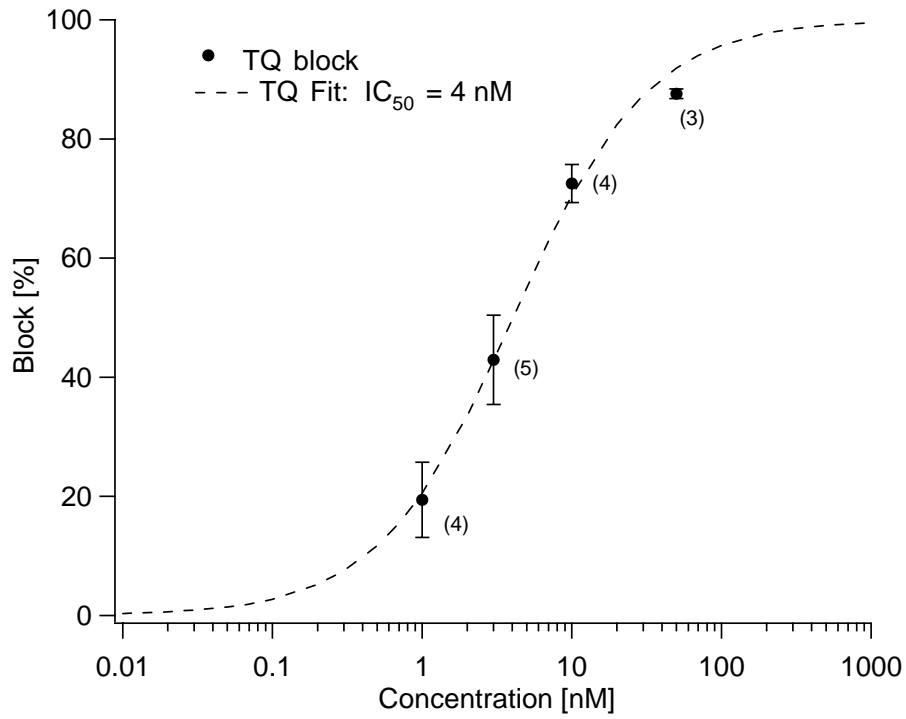


**Figure 1. hK<sub>ir</sub>3.1/K<sub>ir</sub>3.4-HEK Functional Characteristics in Manual Patch Clamp**

**A - B:** Currents (upper panels) elicited by voltage steps from -120 mV to +50 mV in 10 mV increments (lower panels) in transfected (**A**) and untransfected cells (**B**).

**C - D:** Current-voltage relationships. Note the strong inward rectification in the transfected cells (**C**) versus untransfected cells (**D**).

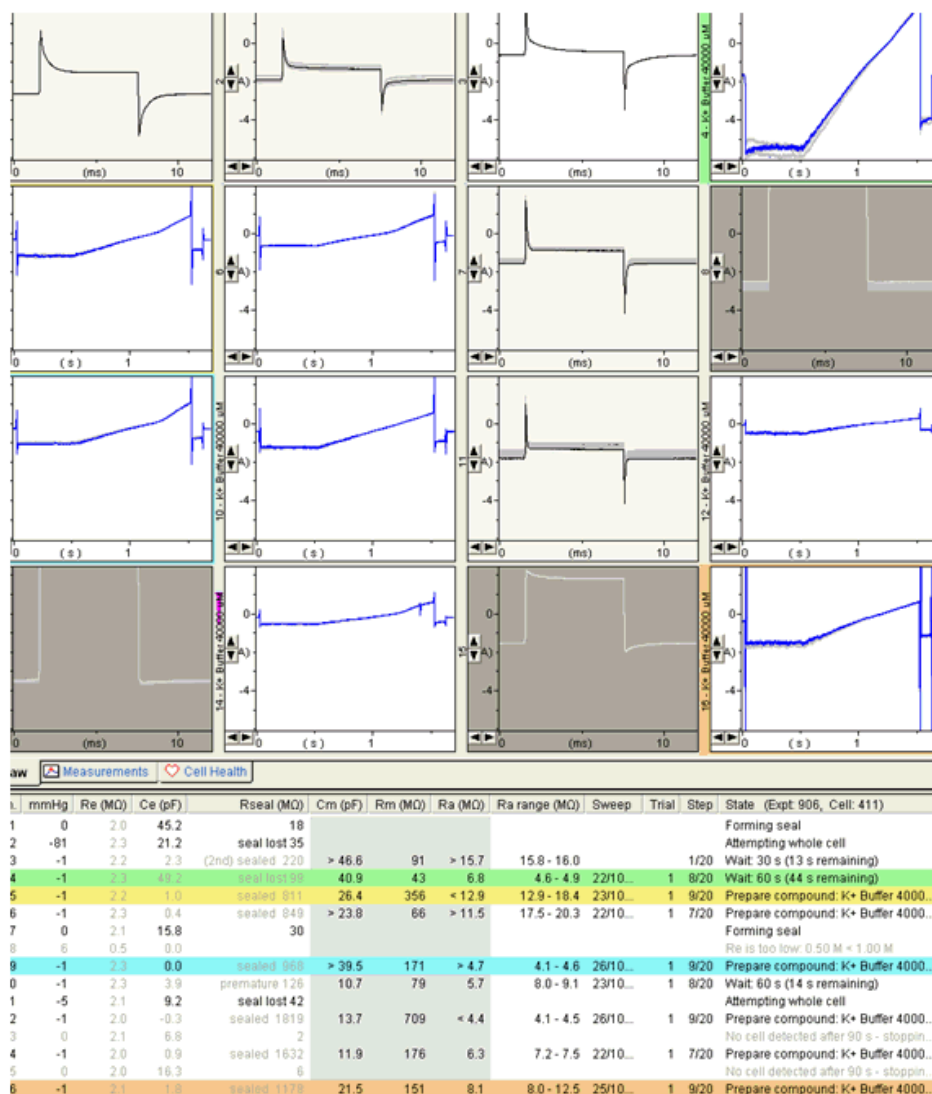
**E - F:** Inhibition of the inwardly rectifying current by 1 mM Ba<sup>2+</sup> in the transfected cell line (**E**). Currents were elicited by a voltage ramp protocol composed of a 500-ms conditioning step to -120 mV from holding potential, -80 mV, followed by a 1-s voltage ramp from -120 mV to +60 mV.



**Figure 2. Tertiapin-Q (TQ) Block in Manual Patch Clamp**  
Concentration-response relationship (Mean  $\pm$  SEM, n = 3 - 5 cells/concentration, IC<sub>50</sub> = 4 nM). Numbers in parentheses denote the number of replicate measurements at each concentration.

## 2.2 PatchXpress<sup>®</sup>

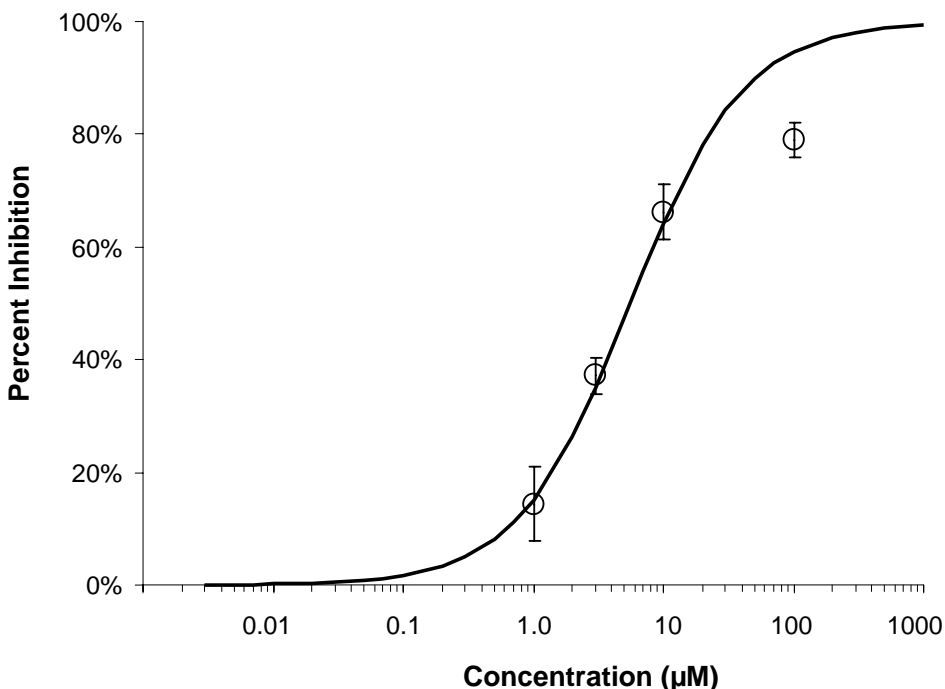
### 2.2.1 Throughput Capability in PatchXpress<sup>®</sup>



**Figure 3. PatchXpress<sup>®</sup> hK<sub>ir</sub>2.1-HEK Screen Capture.**

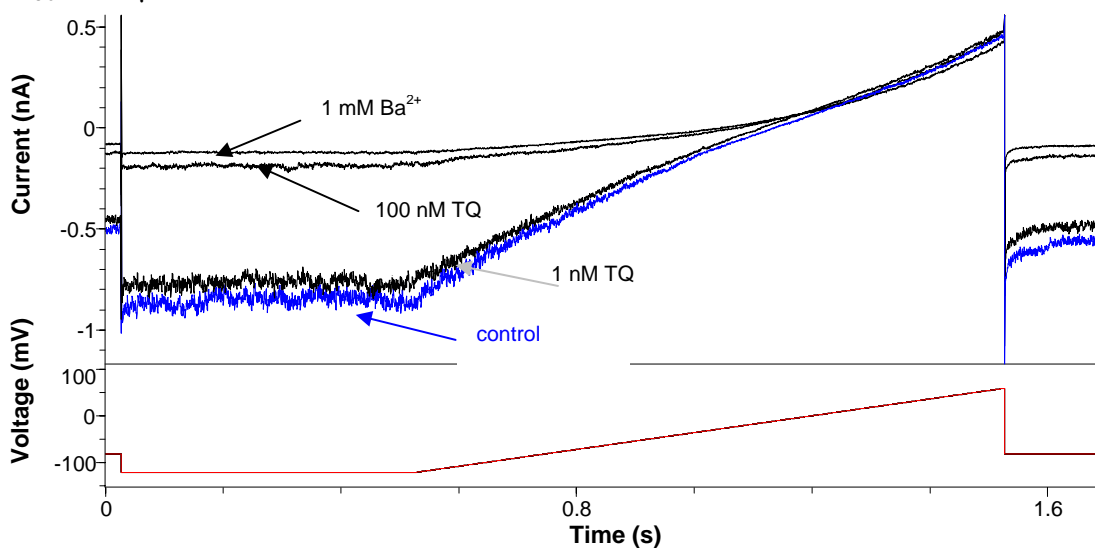
Throughput capability in PatchXpress<sup>®</sup> depends upon many factors which may result in success rate variability. The screen capture shows a typical PatchXpress<sup>®</sup> experiment. In this example, 10 of a possible 16 seals were formed, whole-cell configuration was achieved in 8 cells, and 8 cells showed characteristic hKir3.1/hKir3.4 current waveforms.

### 2.2.2 Representative PatchXpress® Data



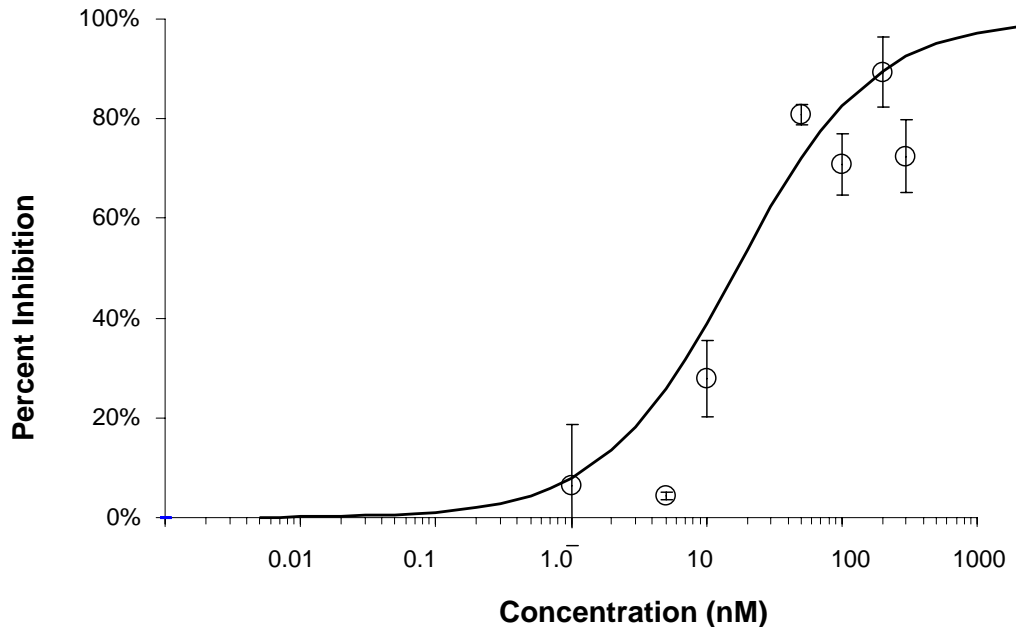
**Figure 4. Concentration-response relationship for Ba<sup>2+</sup> block of hK<sub>ir</sub>3.1/3.4 in PatchXpress®**

The steady state current amplitude before and after Ba<sup>2+</sup> application was used to calculate the percentage inhibition (Mean ± SEM, n = 9 - 11 cells/concentration. IC<sub>50</sub> = 6.8 µM.



**Figure 5. hKir3.1/3.4 current traces in PatchXpress®**

Currents elicited by voltage ramp from -100 to +60 mV, holding potential -80 mV, before (control) and after application of 1 nM tertiapin-Q (TQ), 100 nM TQ and 1 mM Ba<sup>2+</sup>.



**Figure 6. Tertiapin-Q concentration-response relationship in PatchXpress<sup>®</sup>**  
Mean  $\pm$  SEM, n = 2 - 9 cells/concentration. IC<sub>50</sub> = 16.9 nM. Compared with manual patch clamp (Figure 2), the increased variability in these data and the rightward shift in the concentration-response curve is likely due to loss of tertiapin-Q peptide by non-specific binding to the seal chip plastic.

### 3 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.