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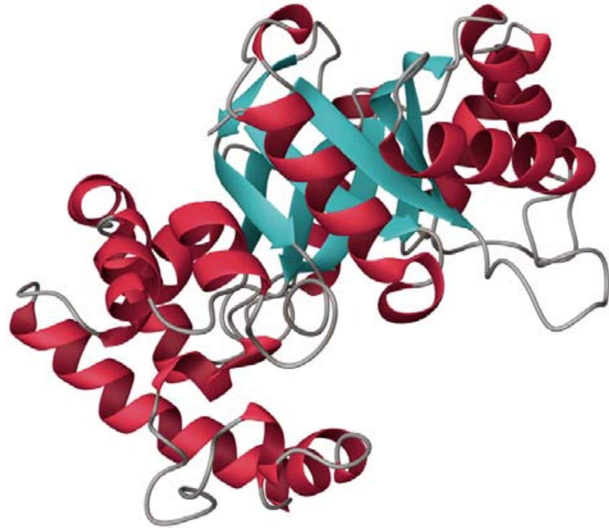
hKv4.3/hKChIP1-CHO K1 Recombinant Cell Line



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Revision 2

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Licensing Statement

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242, USA.

The bovine growth hormone (bgh) polyadenylation signal is patented under U.S. Patent No. 5,122,458. Use, in the USA, of the bgh polyadenylation signal found in screening systems sold by Millipore requires a license from Research Corporation Technologies, Inc. (RCT). After purchasing these materials from Millipore, you must contact RCT within 30 days to obtain a commercial license. The bgh polyadenylation signal cannot be used until a commercial license is obtained. Contact Jennifer Caldwell, Ph.D., at Research Corporation Technologies, Inc., 101 North Wilmot Road, Suite 600, Tucson, AZ 85711-3335, USA Tel: 1-520-748-4400, Fax: 1-520-748-0025.

Provided under license from Wyeth Pharmaceuticals, Collegeville, PA 19426, USA.

This product is covered by the U.S. Patent Nos. 6,361,971, 6,369,197, 6,395,477, 6,689,581, and the U.S. Application Nos. 09/400,492, 09/670,756, 09/703,094, 10/062,879, 10/106,989, 10/118,590.

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Product description:

Recombinant CHO-K1 cell line expressing human Kv4.3 and human KChIP1.

Format:

2 x 1 ml aliquots containing 1.04 x 10^6 cells/ml in 10% DMSO at passage 8.

Mycoplasma Testing:

The cell line has been screened by the PCR VenorGem kit (Minerva Biolabs) to confirm the absence of Mycoplasma species.

Functional Validation:

CHO-K1 cells expressing hKv4.3 and hKChIP1 were characterised in terms of their pharmacological and biophysical properties using whole-cell patch clamp techniques and IonWorks™ HT.

Co-expression of both subunits is strongly supported by the increase in the mean peak currents with respect to expression of Kv4.3 alone (from 9 to 22 nA as recorded using whole-cell patch clamp techniques and from 2.5 to 4.2 nA as measured with IonWorks™ HT).

In addition cells expressing both subunits displayed a more rapid recovery from inactivation (141 ms) with respect to cells expressing just hKv4.3 (1319 ms).

The hKv4.3/hKChIP1 current was inhibited by the antagonists quinidine and flecainide with IC_{50} values of 20 μM and 58 μM respectively.

IonWorks™ HT is a trademark of Molecular Devices Corporation

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Electrophysiological Properties of the hKv4.3/hKChIP1 current.

The Kv4.3 channel is a voltage-gated potassium channel with A-type potassium currents (Gutman *et al.*, 2005). The channel is distributed in the heart, brain and smooth muscle (Serodio *et al.*, 1994; Serodio *et al.*, 1996; Serodio and Rudy, 1998; Gutman *et al.*, 2005). The Kv4 channels contribute to shape the repolarisation of the action potential in the heart (Nerbonne, 2000; Oudit *et al.*, 2001) and in neurones they contribute to the control of the frequency of slow repetitive firing and prevent back-propagation of action potentials (Hoffman *et al.*, 1997; Shibata *et al.*, 2000).
The Kv Channel Interacting Proteins (KChIP) interact specifically with Kv4 channels, upregulating current expression and modulating aspects of channel inactivation (An *et al.*, 2000). KChIP1 increases the current densities of Kv4.3, accelerates the inactivation time course, accelerates the recovery from inactivation and shifts steady-state inactivation to more depolarized potentials (An *et al.*, 2000; Beck *et al.*, 2002; Holmqvist *et al.*, 2002).

Conventional Whole-Cell Patch Clamp Electrophysiology.

Current/Voltage Relationship:

Figures 1 and **2** show potassium currents, evoked by 4 s voltage steps from –80 mV to +50 mV in 10 mV increments, applied from a holding potential of –80 mV in cells expressing hKv4.3/hKChIP1 and hKv4.3 alone respectively. The current/voltage relationships are very similar with activation of the current occurring at voltages more positive than –40/-30 mV in both cell lines. This finding is in agreement with published findings (Beck *et al.*, 2002; Holmqvist *et al.*, 2002; Patel *et al.*, 2004). However, the average current size in the cells expressing hKv4.3/hKChIP1 (22.4 nA) was over twice the size that in the hKv4.3 line (9.1 nA) a result also described by both An *et al.*, 2000 and Holmqvist *et al.*, 2002 co-expressing Kv4.3 and KChIP1 in *Xenopus* oocytes.

Figure 1. Typical hKv4.3/hKChIP1 currents (upper panel) elicited by depolarising voltage pulses from –80 mV to +50 mV in 10 mV increments every 4 s from a holding potential of –80 mV. Scale bars represent 100 ms (x-axis) and 5 nA (y-axis).

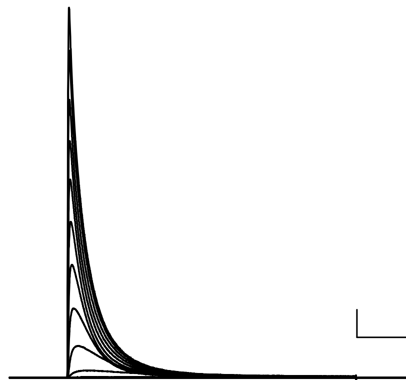

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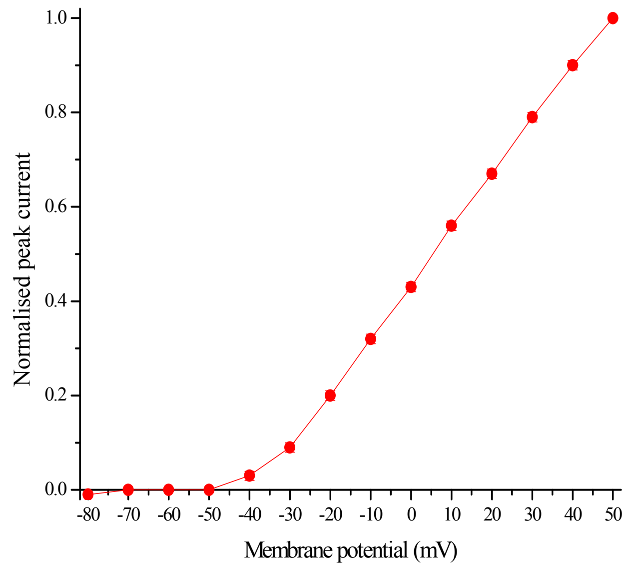
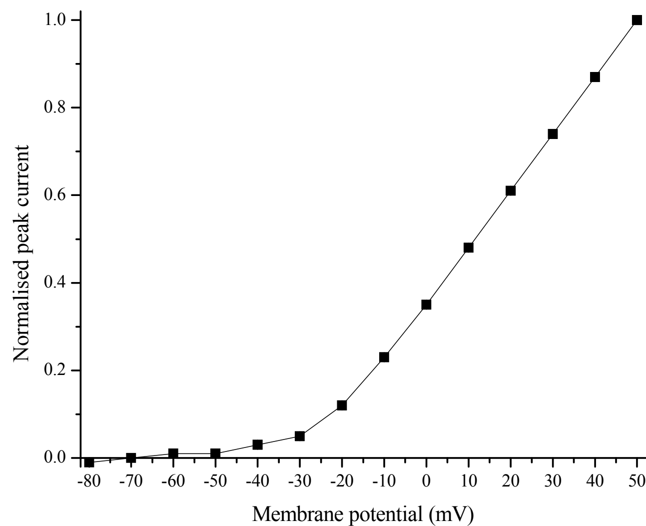
Figure 2. Current-Voltage (I/V) Relationships.
A. hKv4.3/hKChIP1: Peak current amplitudes were normalized to the current amplitude obtained at +50 mV. The mean current at +50 mV was 22.4 ± 6.5 nA (Mean ± SEM, n= 6).
B. hKv4.3: Peak current amplitudes were normalized to the current amplitude obtained at +50 mV. The mean current at +50 mV was 9.1 ± 4.7 nA (Mean ± SEM, n= 7).

A



B



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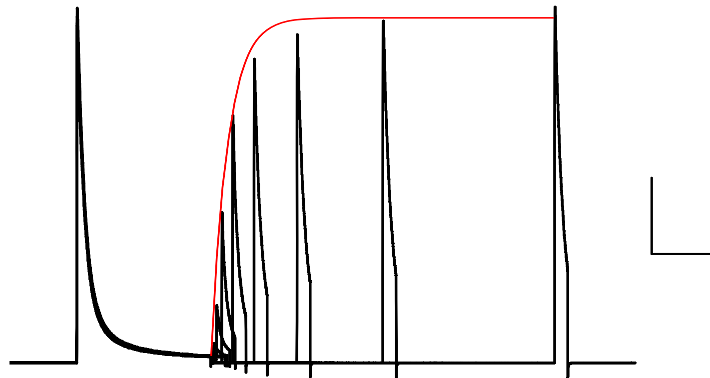
Recovery from Inactivation:

Whilst the I/V relationship is unaffected, the recovery from inactivation is markedly altered by co-expression of the hKChIP1 subunit. **Figure 3A** shows the rapid recovery from inactivation of currents in a cell expressing hKv4.3/hKChIP1. Compare this to the slower recovery when a cell expresses hK4.3 alone (**Figure 3B**). Mean data from several cells is shown in graphical form in **Figure 4**. The value for recovery from inactivation (τ) were 319 ± 29 ms (n=7) and 141 ± 33 ms (n=4) for hKv4.3 and hKv4.3/hKChIP1 respectively (mean ± SEM). Similarly, An *et al.*, 2000, Holmqvist et al., 2002 and Beck et al., 2002 found that co-expression of the subunit speeds up the recovery from inactivation (τ=120-327 ms when expressing hKv4.3 alone and 34.5-63 ms when expressing hKv4.3/hKChIP1, mean ± SEM, all data from *Xenopus* oocytes).

Figure 3.
A. hKv4.3/hKChIP1: Currents evoked by stepping from holding potential of –80 mV to 50 mV for 1 s for the 1st pulse and for 100 ms for the second pulse. Scale bars represent 500 ms and 5 nA. Red line is single exponential fit to peak of 2nd pulse peak currents. Second pulses after a interval of 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2560 ms.



B. hKv4.3: Currents evoked as above. Scale bars represent 500 ms and 1 nA. Red line is single exponential fit to peak of 2nd pulse peak currents. Second pulses after a interval of 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2560 ms.

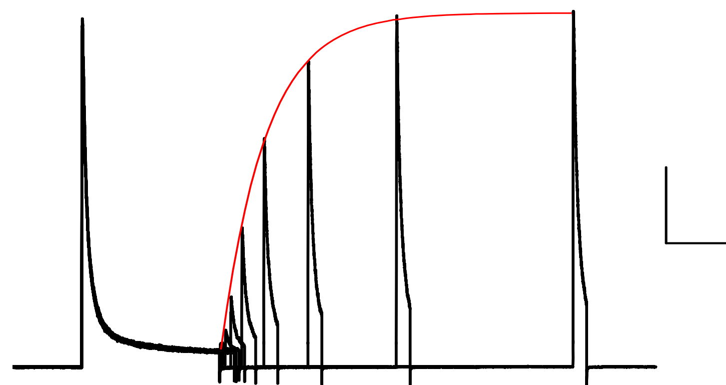


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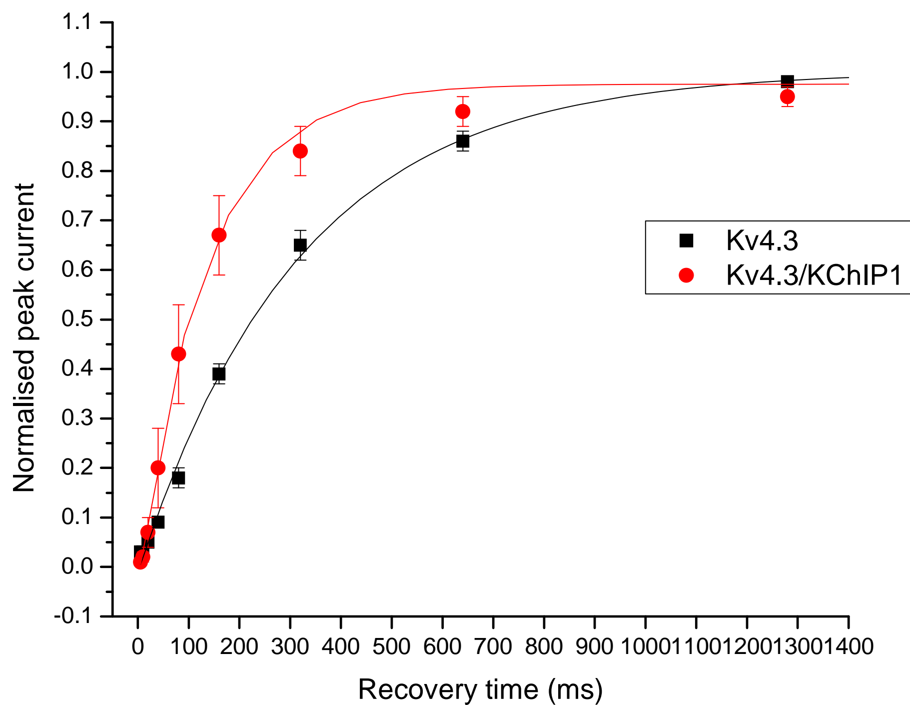
Figure 4. Recovery of currents from inactivation.
Normailised peak currents (y-axis) are plotted against the recovery time (x-axis). hKv4.3 alone (black) and hKv4.3 coexpressed with hKChIP1 (red). The values for recovery from inactivation (τ) were 319 ± 29 ms (n=7) and 141 ± 33 ms (n=4) for hKv4.3 and hKv4.3/hKChIP1 respectively (mean ± SEM).



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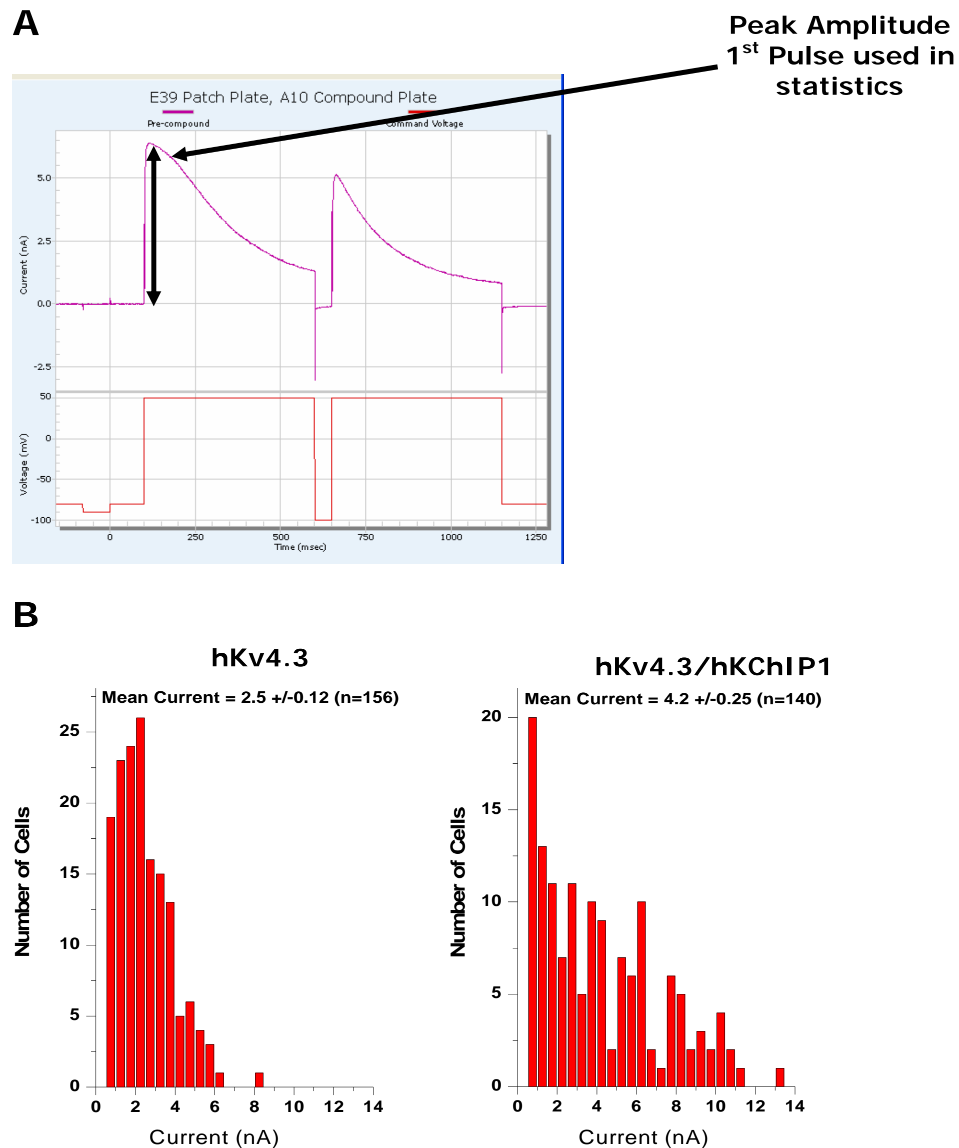
IonWorks™ HT Electrophysiology.

Comparison of peak current amplitudes in hKv4.3/hKChIP1-CHO K1 and hKv4.3 -CHO K1 cell lines:

Both cell lines were studied using the same double-pulse voltage protocol shown in **Figure 5A** (red trace). A typical current response elicited by this protocol is shown in magenta. The results of this comparison are shown in a histogram in **Figure 5B**. The co-expression of hKv4.3 with hKChIP1 increased the mean peak current amplitude from 2.5 ± 0.12 nA (n=156) to 4.2 ± 0.25 (n=140) (mean ± SEM). These values reflect the increase in mean current values, obtained by conventional whole-cell patch clamp electrophysiology, from 9.1 nA to 22.4 nA in cells expressing hKv4.3 and hKv4.3/hKChIP1 respectively.

Figure 5.
A. Current responses and voltage protocol.
B. Current amplitude distributions.



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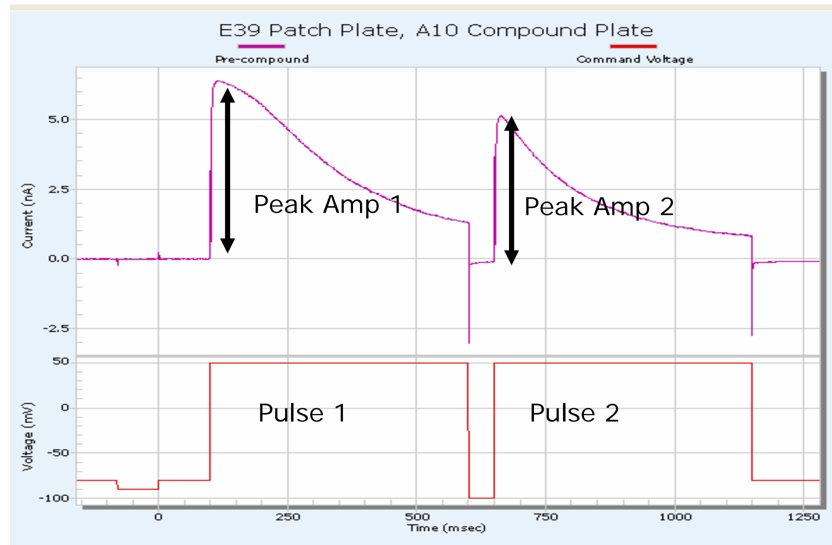
Recovery from Inactivation:

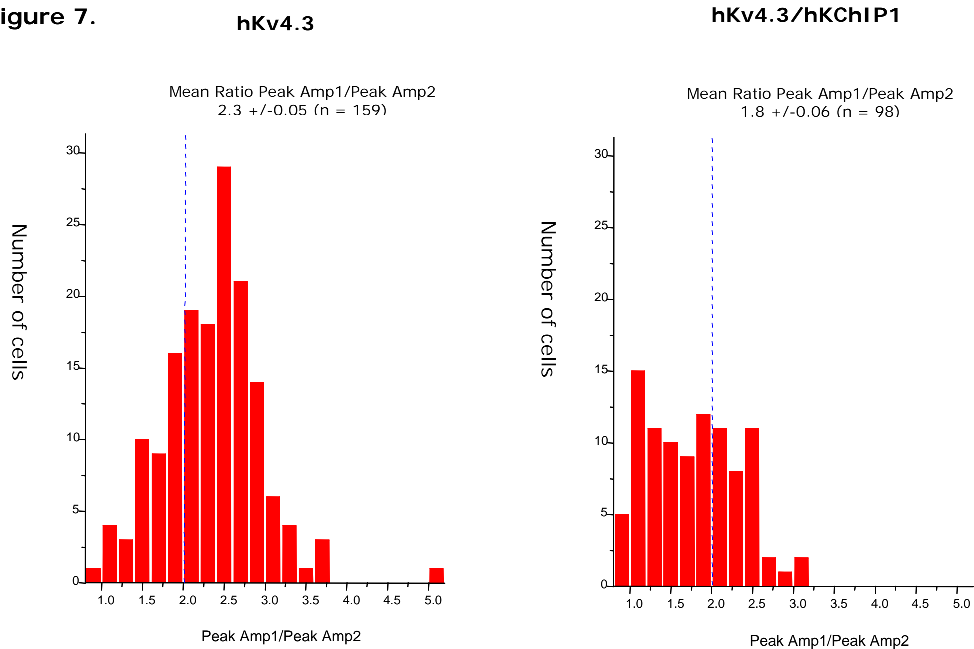
The recovery of the current from inactivation was also studied on IonWorks™ HT using the same double pulse voltage protocol as before and shown again in **Figure 6** (red trace). In order to quantify the difference in the degree of recovery from inactivation between the two cell lines, the following metric (Peak Amp1 / Peak Amp2) was applied to the currents obtained by pulses 1 and 2 (**Figure 6**).

Figure 6.



In agreement with the data from the conventional whole-cell patch clamp experiments, the currents in the hKv4.3/hKChIP1-CHO K1 cell line display a more rapid recovery from inactivation than the hKv4.3-CHO K1 currents and therefore have a ratio closer to 1.

Figure 7. **hKv4.3** **hKv4.3/hKChIP1**



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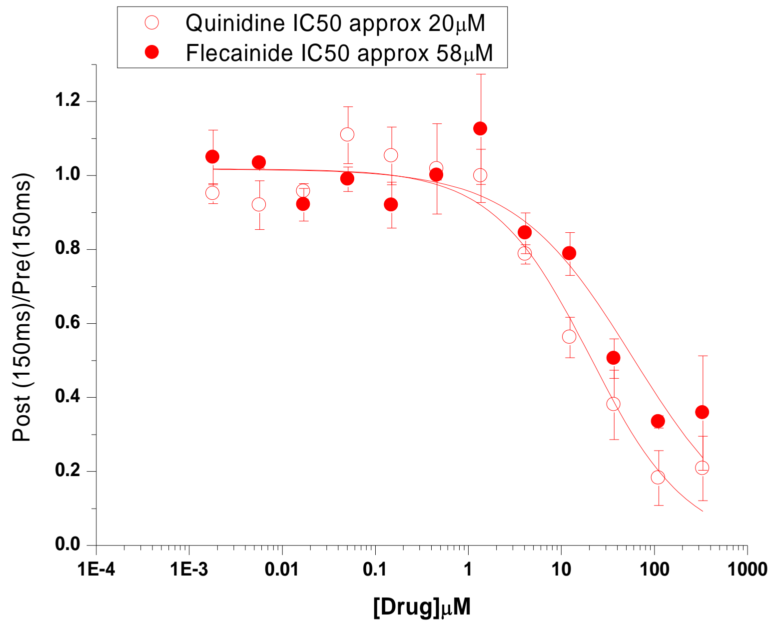
Pharmacology – quinidine and flecainide:

The antagonists quinidine and flecainide are both known antagonists of Kv4.3 currents. In these studies, the pre-compound and post-compound currents elicited by the double pulse protocol were recorded and the post-/pre-compound ratio plotted against antagonist concentration (**Figure 8**).

Figure 8. Dose-response curves for the reduction in post-/pre-compound ratio by the antagonists quinidine and flecainide.



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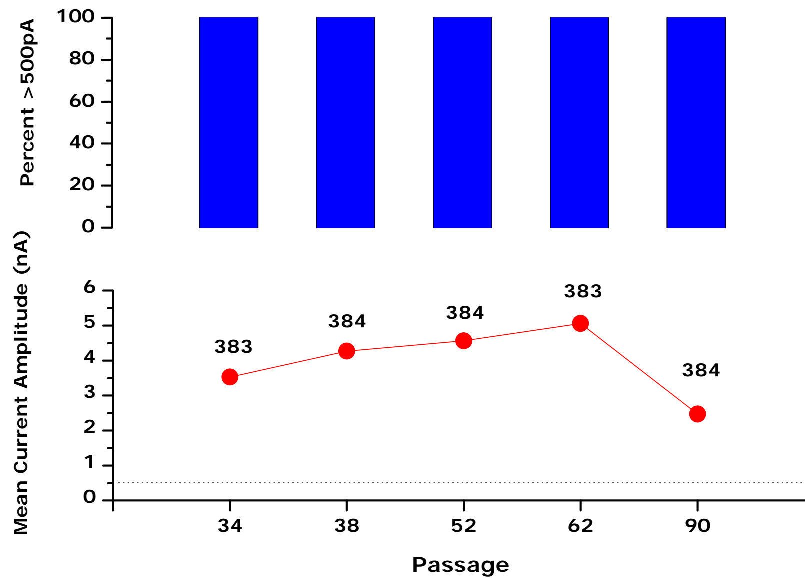
Stability of hKv4.3/hKChIP1-CHO K1 Cell Line:
The hKv4.3/hKChIP1-CHO K1 cell line has stable expression for >90 passages.

Figure 9. Stability of expression over passage.
The upper panel shows the percentage of cells expressing a mean peak current >500 pA at cell passages 34, 38, 52, 62, and 90. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles).



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Recommended Culture Conditions:

Cells should be grown in a humidified environment at 37°C under 5% CO_2 using D-MEM with Glutamax medium supplemented, 10% Foetal Bovine Serum, 1% Non Essential Amino Acids, plus 500 μg/ml of Geneticin and 500 μg/ml Zeocin to ensure that the recombinant expression is maintained.

Transfection of CHO-K1 host cells with the human Kv4.3 and KChIP1 constructs did not appear to alter the growth characteristic of the host cells, which exhibited a normal cell division time of approximately 16 hours.

It is recommended to rapidly thaw a frozen aliquot from liquid nitrogen by agitation in a 37°C water-bath and to transfer vial contents into a T175 cm^2 flask containing 30 ml of pre-equilibrated media according to the formulation below. Allow cells to adhere for 4-8 hours at 37°C under 5% CO_2 before gently removing the media and replacing with 30 ml of fresh media.

The cell line should not be allowed to exceed 70% confluency within the culture vessel, to prevent contact inhibition causing senescence. Passage every 2-3 days by rinsing with phosphate buffered saline before harvesting with Trypsin/EDTA and seeding into new flasks using a seeding density of $0.5\text{-}1\times10^6$ cells per T75 cm^2 or $1\text{-}2\times10^6$ cells per T175 cm^2 flask. It is essential that the cell line is continually maintained in the presence of Geneticin (500 μg/ml) and Zeocin (500 μg/ml), which should be added to the culture media immediately prior to use.

Media Formulation:

D-MEM (with GlutaMAX™ I)	(Invitrogen	#31966)
10% Foetal Bovine Serum	(Invitrogen	#16000)
1% Non Essential Amino Acids	(Invitrogen	#11140)
500 μg/ml Geneticin	(Invitrogen	#10131)
500 μg/ml Zeocin	(Invitrogen	#45-0430)

Other reagents required:

Trypsin/EDTA	(Invitrogen	#25300)
PBS	(Invitrogen	#14190)
Trypan Blue	(Sigma	#T8154)
DMSO	(Sigma	#D2650)

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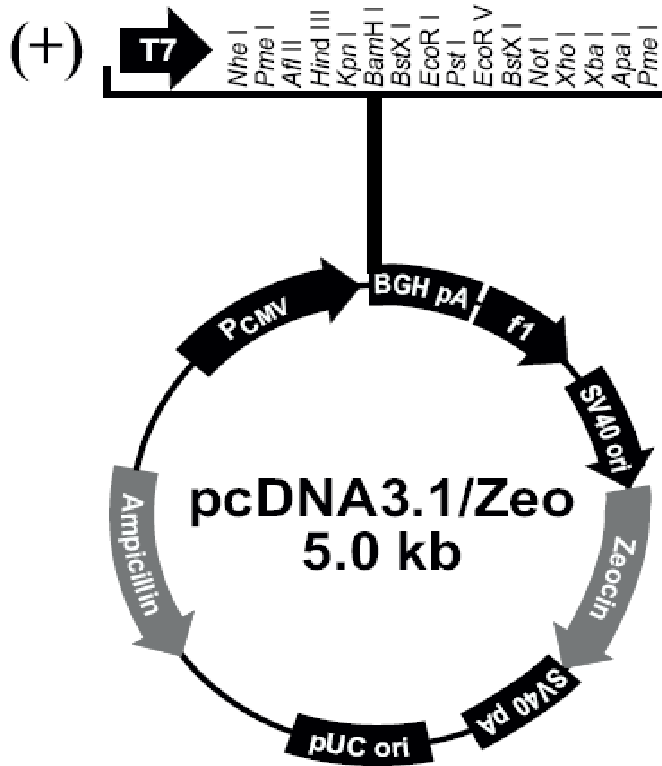
Vectors:

hKv4.3-pcDNA3.1/zeo(+)



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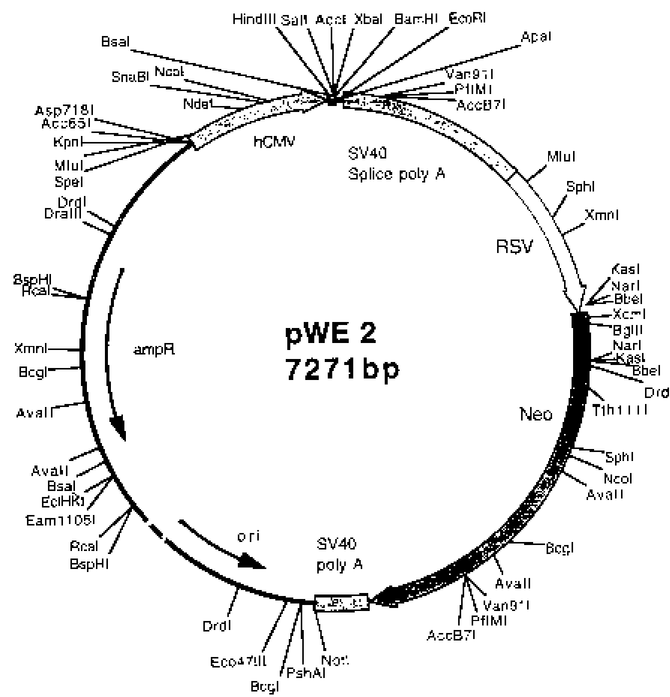
hKChIP1-pWE2



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hKv4.3 Sequence:

The sequence of the cDNA used to make this cell line contains two silent mutations and two coding mutations (GCC-ACC (Ala-Thr) and GCC-GTC (Ala-Val)) with respect to the accession number NM_004980.

```
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hKChIP1 Sequence (Accession Number NM_014592):

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