hKv7.2/hKv7.3-CHO K1 (hKCNQ2/hKCNQ3) Recombinant Cell Line
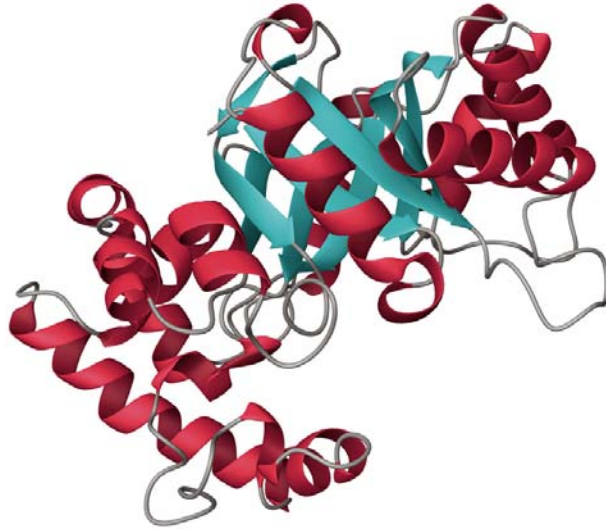
cat. #CYL3059

Revision 1

Ordering Information and Technical Services:

MILLIPORE (UK) LIMITED
6-7 Technopark
Cambridge
CB5 8PB
UK
Tel: +44 (0) 1223 508191
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Customer Services UK: 0800 0190 333
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Contents:

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.

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Product description:

Recombinant CHO-K1 cell line co-expressing the human Kv7.2 [voltage-gated potassium channel, KQT-like subfamily, member 2 (KCNQ2), accession number Y15065] and the human Kv7.3 [voltage-gated potassium channel, KQT-like subfamily, member 3 (KCNQ3), accession number NM_004519].

Format:

2 x 1 ml aliquots containing 1.58×10^6 cells/ml in 7.5% DMSO at passage 10.

Mycoplasma Testing:

The cell line has been screened using the PCR Mycoplasma Test Kit (MDBiosciences) to confirm the absence of Mycoplasma species.

Functional Validation:

hKv7.2/hKv7.3 (KCNQ2/KCNQ3) channels have been selectively expressed in a CHO-K1 cell line and characterized in terms of their biophysical and pharmacological properties using whole-cell and perforated patch clamp techniques and IonWorks™ Quattro.

The currents displayed all the hallmarks for selective expression of Kv7.x channels such as a relatively negative threshold of activation, the absence of any inactivation and slow activation/deactivation kinetics. The current/voltage relationship was linear and the mean outward current at 0 mV was 2.2 ± 0.3 nA (n=3). Thus this cell line has an ideal level of functional expression for both manual and automated patch clamp applications.

Using perforated patch clamp techniques the currents were found to be dose-dependently blocked by TEA in the appropriate range for expression of Kv7.2/Kv7.3 channels (approximately 58% inhibition at 3 mM). The effects of the selective Kv7.x blockers linopirdine and XE991 were also assessed using IonWorks™ Quattro and the IC_{50} values obtained were similar to published values; around 9 μM and 2 μM respectively.

Channel expression, monitored using IonWorks™ HT, is robust over at least 40 passages. For example 96% of cells expressed outward current >500 pA at passage 40 (n=170) with a mean current amplitude of 2 +/-0.06 nA.

IonWorks™ HT and IonWorks™ Quattro are trademarks of Molecular Devices Corporation

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Introduction:

The predominant heteromultimer responsible for the neuronal M-current is now known to consist of hKv7.2 (KCNQ2) and hKv7.3 (KCNQ3) subunits (Wang *et al.,* 1998). This current was first described in bullfrog sympathetic neurons (Brown and Adams, 1980) and subsequently found to be distributed in various central and peripheral neurons. It has a fundamental role in determining the sub-threshold excitability of neurons and their responsiveness to synaptic input (Cooper, 2001 and Jentsch, 2000). This is highlighted by the observation that mutations that reduce the number of functional Kv7.2/Kv7.3 channels can result in an inherited form of epilepsy (Chung *et al.,* 2006). Since these channels have such an important role in regulating neuronal excitability they have become important targets for the treatment of epilepsy and pain.

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Electrophysiological Properties of the hKv7.2/hKv7.3 Current.

Conventional Whole-Cell Patch Clamp Electrophysiology.

hKv7.2/hKv7.3 kinetics:
hKv7.x channels have a number of characteristic electrophysiological properties which, taken together, make them distinct from other Kv channels. These are a relatively negative threshold of activation (-50/-60 mV), slow activation and deactivation kinetics and the absence of inactivation. These distinctive kinetic properties are illustrated in **Figure 1A**. The membrane voltage was stepped from a holding potential of -60 mV to -30 mV which evoked, after a significant delay (**Figure 1A**, arrow), a slowly activating outward current that was sustained for the duration of the depolarizing pulse. The delay in onset and sustained activation are typical of both the native M-current and Kv7.2/Kv7.3 expressed in mammalian cell lines (Wang *et al.*, 1998 and Adams and Brown, 1982). Stepping the membrane voltage back to -60 mV first caused an instantaneous reduction in the outward current, reflecting the reduced driving force for potassium through open channels activated at -30 mV, followed by a slowly deactivating tail current. Inverting the voltage protocol by using a holding potential of -30 mV and stepping to -60 mV, produced the expected current trace for non-inactivating M/Kv7.2/Kv7.3 currents reported previously (Adams and Brown, 1982). For example, holding the potential at -30 mV produced a large non-inactivating outward current (around 1 nA in **Figure 1A**) that deactivated slowly at -60 mV after the instantaneous current. Subsequently returning to -30 mV after all the channels had closed at -60 mV, lead to re-activation of the current along the expected sigmoidal time course, after the initial delay.

To further characterise the time course of deactivation, the voltage was first stepped to -30 mV for 2 s to fully activate the channels prior to returning to various test potentials (**Figure 1B**). The time course of current deactivation at each test potential was measured by fitting the current to an exponential. Time constants for the fits were 73 ± 9.5 ms, 113 ± 20 ms, 172 ± 29 ms and 205 ± 37 ms for -70 mV, -60 mV, -50 mV and -40 mV respectively (n =3). These values are similar to values obtained in sympathetic neurons, e.g. around 44 ms and 150 ms at -70 mV and -40 mV respectively (Adams and Brown, 1982).

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Figure 1. Kinetics of hKv7.2/hKv7.3 currents.
A. hKv7.2/hKv7.3 currents were either opened by a depolarizing step from -60 mV to -30 mV (upper panel) or closed by a hyperpolarizing step from -30 mV to -60 mV (lower panel).
B.   The time course of current deactivation was assessed by stepping the membrane voltage from -30 mV to various negative potentials (lower panel). Decaying currents (upper panel) were fitted with exponentials.

A

B
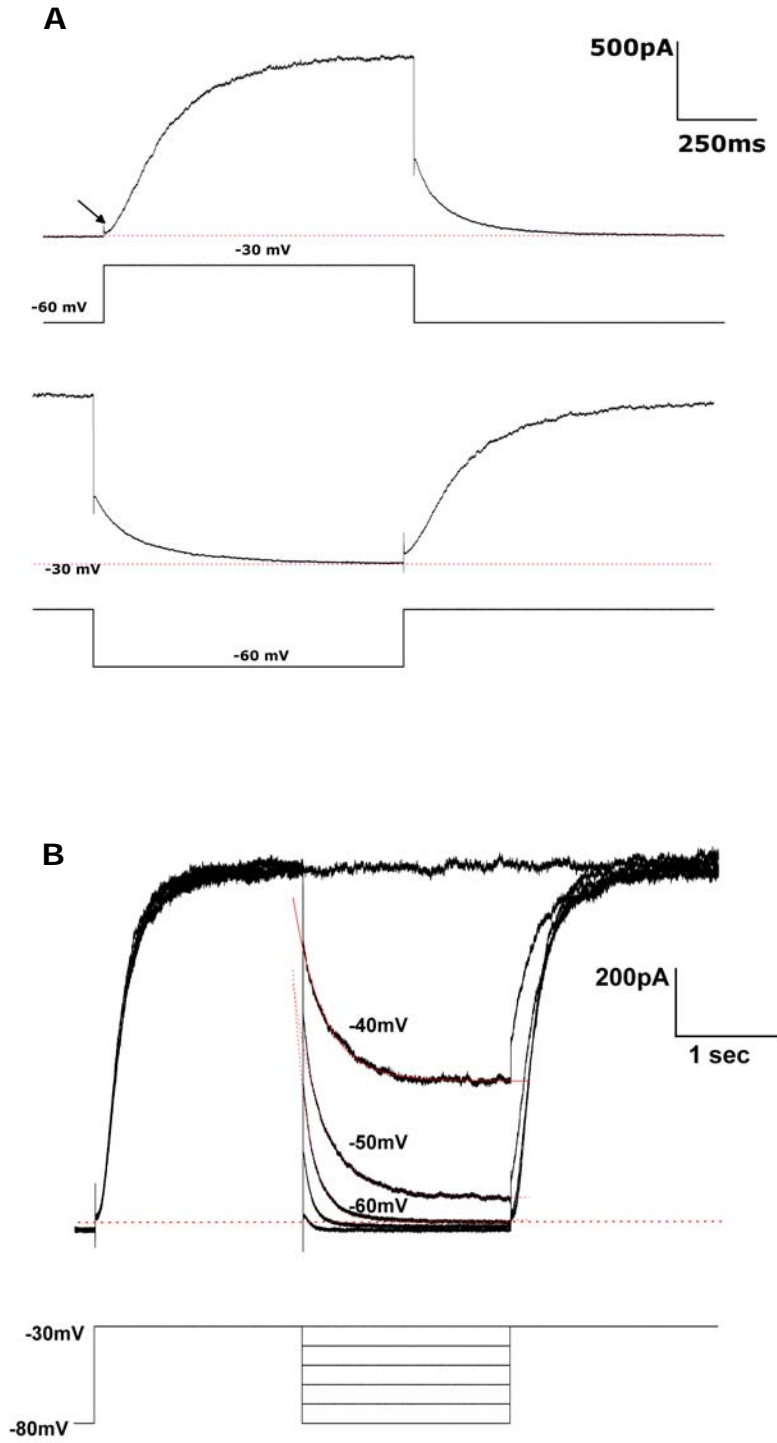
Current-voltage (I/V) relationship:
The I/V relationship was examined by stepping from a holding potential of   -80 mV to increasingly depolarized voltages for 2 s; -80 mV to +50 mV in 10 mV increments every 10 s (**Figure 2**). As described above, currents activated subsequent to a delay and slowly reached a sustained level during the depolarizing pulse, with no inactivation (**Figure 2A**). The I/V plot was obtained by measuring the amplitude of the current at the end of the depolarizing step and plotting these values against the step voltage (**Figure 2B**). The threshold of activation was quite negative, between –50 mV and -40 mV, which is another distinguishing feature of this K^+ channel (see above). Similar to previously published data (Main *et al.,* 2000, Zhang *et al.,* 2003) the I/V relationship was linear with a mean sustained outward current of 2.2 ± 0.3 nA (n=3) at 0 mV.

Figure 2. I/V relationship of hKv7.2/hKv7.3 currents.
A. Currents (upper panel) were evoked by 2000 ms depolarising voltage pulses stepped in 10 mV increments from -80 mV to +50 mV from a holding potential of –80 mV once every 10 seconds (lower panel). The green dotted line indicates zero current level.
**B** The steady state current amplitudes elicited by the voltage protocol shown in **A** were normalised to the current evoked by the +50 mV voltage step for each cell. The mean data (n=3) is shown plotted against the step potential (mV).
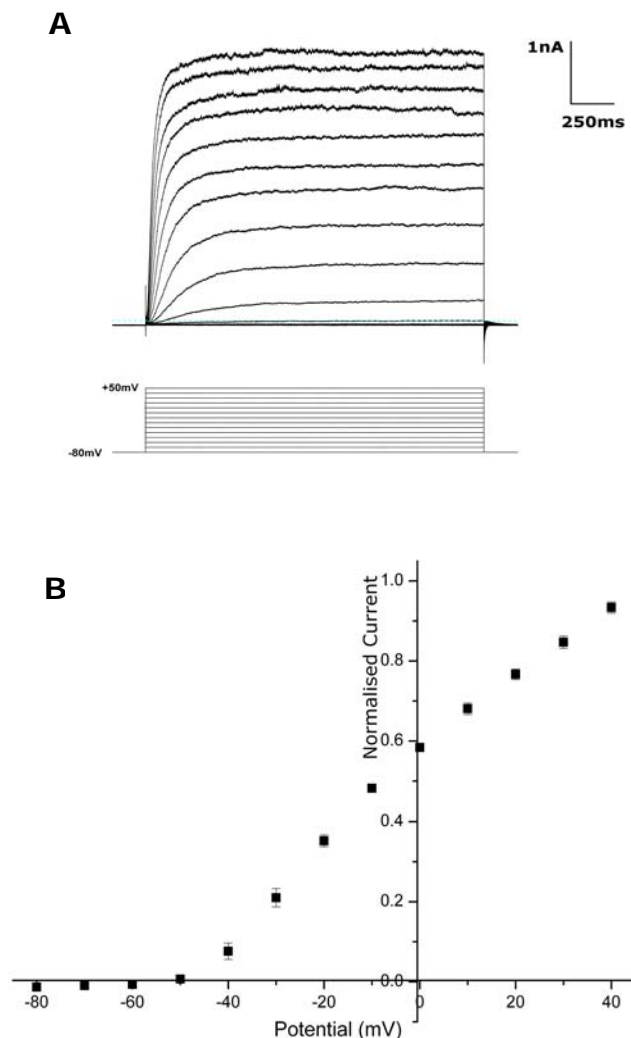
Kv7.x currents, unlike many other Kv channels have a marked tendency to 'run-down' in whole-cell mode. The rate and extent of this 'run-down' varies from cell to cell but can be largely removed by employing the perforated patch technique (Guo and Schofield, 2002 and Hadley *et al.,* 2000). This is illustrated in **Figure 3** where hKv7.2/hKv7.3 currents were evoked by repetitive pulses to 0 mV every 10 s. The amplitude of each pulse is plotted against pulse number from the time of either establishing whole cell (solid circles) or perforated patch with an access resistance <15 MOhm (open circles). The current has a consistent amplitude of around 2 nA in the perforated patch configuration but 'runs down' in whole-cell mode. Consequently it is recommended that wherever possible the perforated patch technique is employed when using this cell line.

Figure 3. I/V relationship of hKv7.2/hKv7.3 currents.
hKv7.2/hKv7.3 currents were evoked every 10 s by voltage steps from a holding potential of -80 mV to 0 mV. Each evoked current was plotted against pulse number from the time of establishing whole-cell (solid circles) or the perforated patch configuration (open circles).
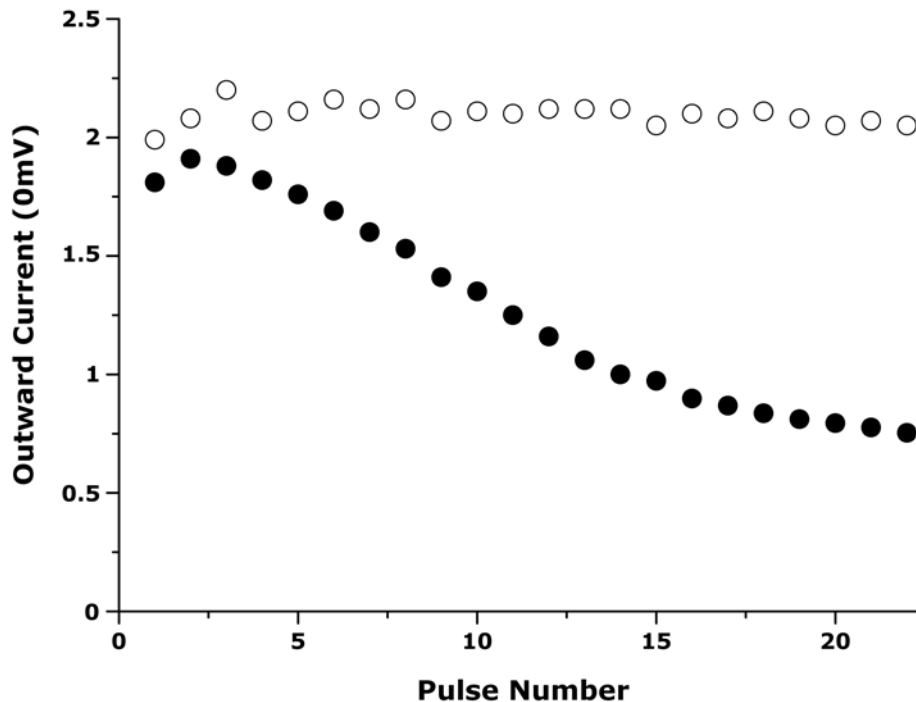
Pharmacology - Tetraethylammonium chloride (TEA).

Manual Patch Clamp (Perforated Patch Configuration).

TEA has previously been used as a pharmacological tool to discriminate between the various combinations Kv7 subunits (Wang *et al.,* 1998, Hadley *et al.,* 2000). The differential sensitivity is conferred by the variable presence of tyrosine, thereonine or valine in the upper pore region, downstream from the GYG selectivity sequence. Pertinent to this study it was reported (Hadley *et al.,* 2000) that in a recombinant CHO cell line homomeric Kv7.2 channels are the most sensitive (IC_{50} = 0.3 mM), Kv7.3 channels are relatively insensitive (IC_{50} >30 mM) whereas heteromultimers of Kv7.2/Kv7.3 channels have an intermediate sensitivity (IC_{50} ~ 4mM). Consistent with the presence of heteromulitmers, bath application of 0.3 mM, 3 mM and 30 mM TEA blocked currents by 28 ± 2.9% (n =5), 57.5 ± 8% (n = 6) and 88.3 ± 5.5% (n = 6) respectively (**Figure 4**).

Figure 4. **Effect of TEA on hKv7.2/hKv7.3 currents**.
A. Cells were pulsed to a potential of 0 mV for 2 seconds from a holding potential of -80 mV and repeated every 10 seconds. Once stable hKv7.2/hKv7.3 current amplitudes at 0 mV were achieved under control conditions, increasing doses of TEA were cumulatively applied to the cell, allowing each concentration to achieve a stable reduction in current amplitude prior to addition of a subsequent dose. Outward current measured at the end of the depolarizing pulse to 0 mV is plotted against time.
B. Typical current records obtained prior to addition (Control, black trace) and after 0.3 mM (red trace), 3 mM (blue trace) and 30 mM (green trace) TEA. The blue dotted line is the zero current level.
C. The mean percent inhibition relative to the control amplitude prior to addition for each concentration of TEA is shown (n = 5-6).

A

B

C

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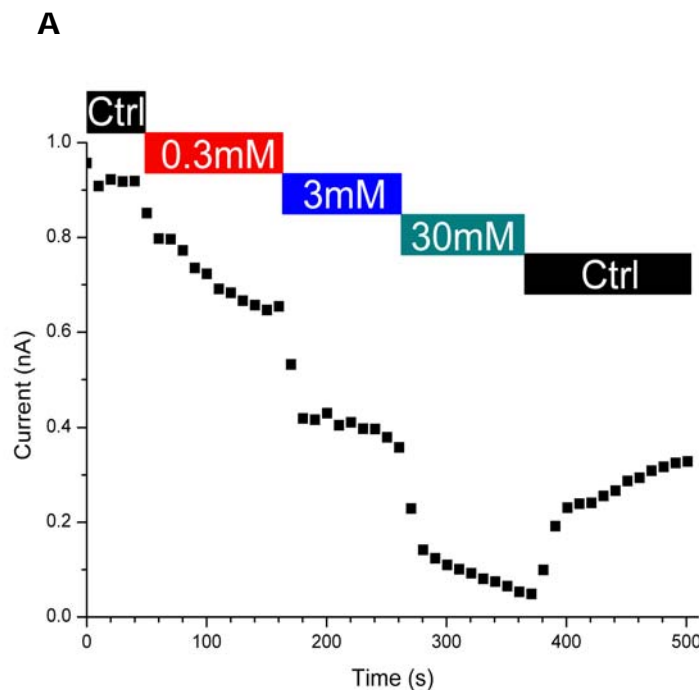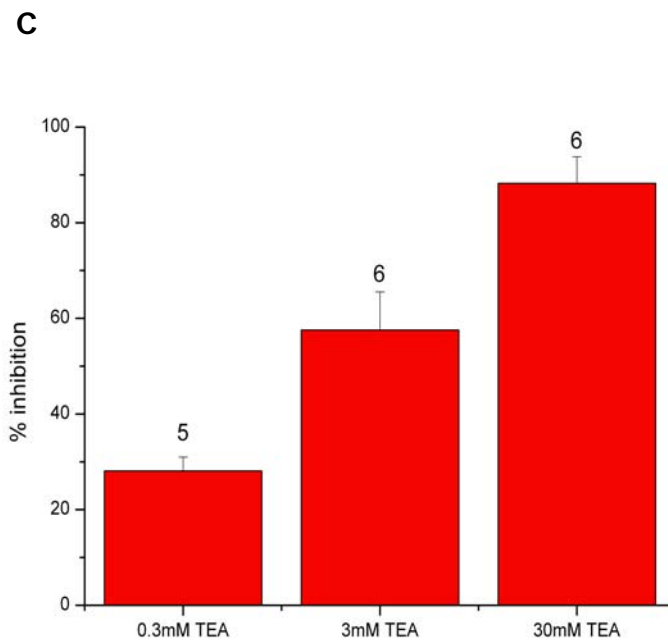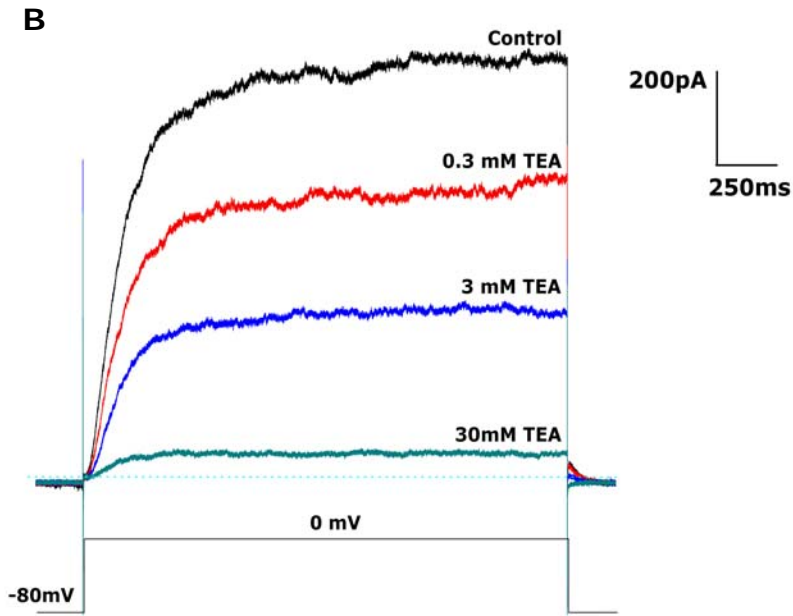
Pharmacology – Linopirdine and XE991.

IonWorks^{TM} Quattro (Population Patch Configuration).

The cognitive enhancer linopirdine and the more potent analogue, XE991 have frequently been used in the characterization of Kv7.x channels. Reported IC_{50} values for linopirdine for the native M-current range between 2–7 µM (Passmore *et al.,* 2003, Wang *et al.,* 1998, Costa and Brown, 1997; Lamas *et al.,* 1997) and for Kv7.2/Kv7.3 currents around 4 µM (Wang *et al* 1998). Consistent with these findings linopirdine dose-dependently blocked hKv7.2/hKv7.3 currents with an estimated IC_{50} of 8.5 µM (**Figure 5A**). Reported IC_{50} values for XE991 range between 0.3-1 µM for the native M-current (Passmore *et al.,* 2003; Wang *et al.,* 1998) and 0.6 µM for Kv7.2/Kv7.3 currents (Wang *et al.,* 1998). Here, the IC_{50} value for XE991 block was around 2.2 µM (**Figure 5B**). This slight discrepancy with previous reports (2-4 fold) is most likely due to this method of recording.

Figure 5. Effect of Linopirdine and XE991 on hKv7.2/hKv7.3 currents.
The effect of a 10 min incubation of various concentrations of either linopirdine (**A**) or XE991 (**B**) was assessed on the amplitude of hKv7.2/hKv7.3 currents using IonWorks^{TM} Quattro automated electrophysiology. Each data point represents the mean of 12 cells.

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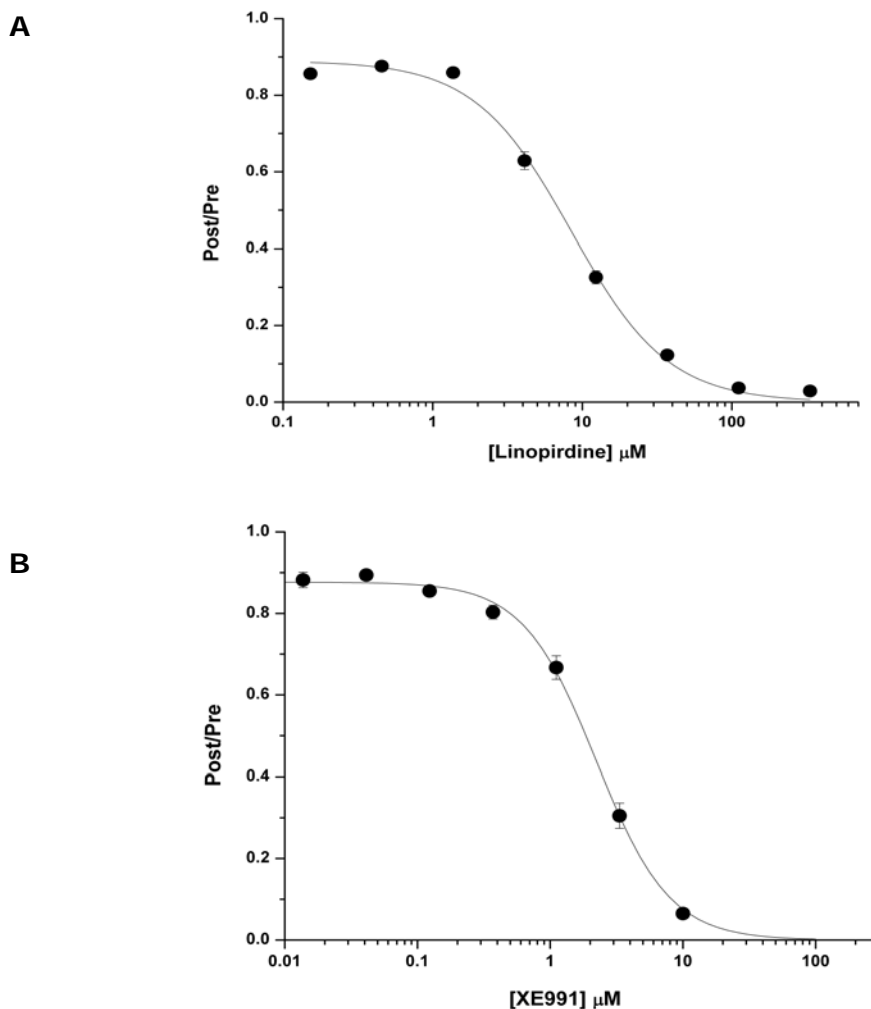
Stability of hKv7.2/hKv7.3-CHO K1 Cell Line.

IonWorks™ HT Electrophysiology.

The hKv7.2/hKv7.3-CHO K1 cell line has stable expression for >40 passages.

Functional channel expression, defined as cells expressing potassium current of > 500 pA, was monitored using IonWorks™ HT. This data and the mean current amplitude is shown in **Figure 6**. Sealing data is shown in **Figure 7**.

Figure 6. Stability of expression over passage.
The upper panel shows the percentage of cells expressing a mean peak current >500 pA at 0 mV at cell passages 10, 19, 27, 31, 37 and 40. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of cells (numbers above red circles - out of 64 cells for passage 10 and out of 192 cells for all other passages).
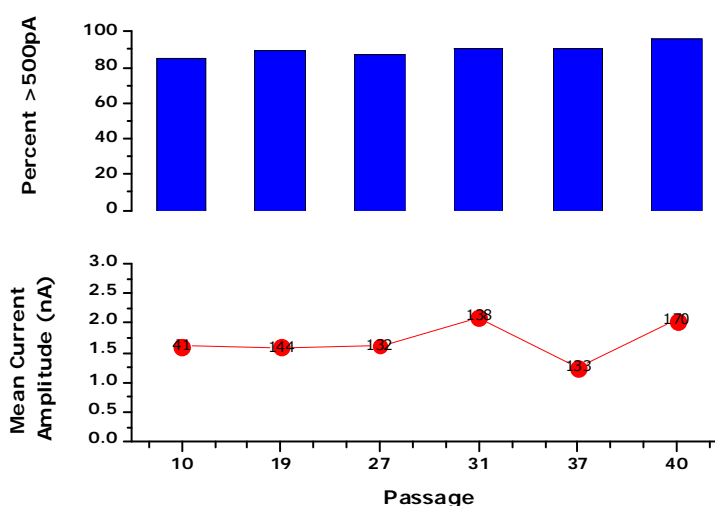
Figure 7. Sealing rates over passage.
The percentage of cells sealing (defined by a seal resistance of >50 MΩ).

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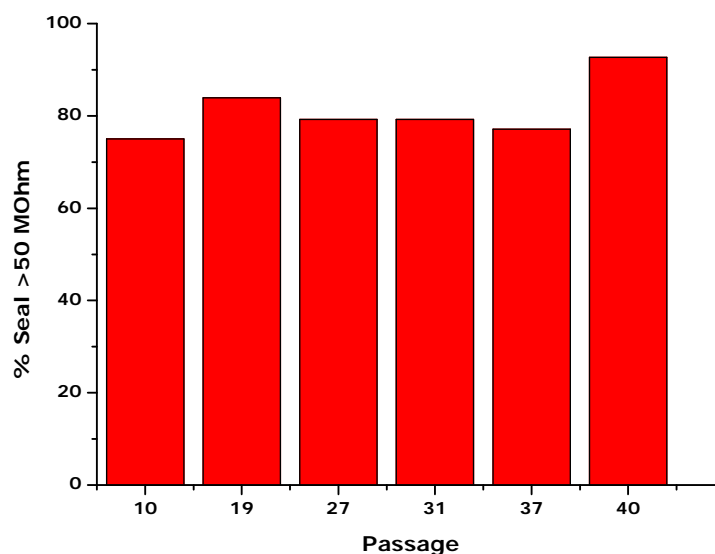
Recommended Culture Conditions:

Cells should be grown in a humidified environment at 37°C under 5% CO_2 using F-12 Nutrient Mixture (Ham) (with GlutaMAX^{TM} I), 10% FBS, plus 400 µg/ml of Geneticin and 100 µg/ml Hygromycin B and to ensure that the recombinant expression is maintained.

Transfection of the CHO K1 cells with the hKv7.2 and hKv7.3 ion channels does not appear to have altered the growth characteristics of the host cells which exhibited a typical cell division time of 16 hours.

It is recommended to quickly thaw a frozen aliquot from liquid nitrogen, by agitation in a 37°C water-bath, before transferring into a T175 cm^2 flask containing 50 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 80% confluency within the culture vessel, to prevent contact inhibition causing senescence and should thus be passaged every 2-3 days using a seeding density of 0.5-1$\times10^6$ cells per T75 cm^2 or 1-2$\times10^6$ cells per T175 cm^2 flask. Pre-washing with phosphate buffered saline before harvesting with Trypsin/EDTA and seeding into new flasks is recommended to passage the cell line. It is essential that the cell line is continually maintained in the presence of Geneticin (400 µg/ml) and Hygromycin B (100 µg/ml), which should be added to the culture vessel or media immediately prior to use.

Media Formulation:

F-12 Nutrient Mixture (Ham) (with GlutaMAX^{TM} I)	(Invitrogen	#31765)
10% Foetal Bovine Serum	(Invitrogen	#16000)
400 µg/ml Geneticin	(Invitrogen	#10131)
100 µg/ml Hygromycin B	(Invitrogen	#10687)

Other reagents required:

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

Vectors:

Plasmid map of **hKv7.2-pCIN5**, 4904 bps.

Labeled features and restriction sites:

HincII, SspI, MluI, HincII, NdeI, SnaBI, PvuI, CMV promoter, Amp, AhdI, BamHI, ApaI, 4000, 1000, IRES, 3000, 2000, Neo, PstI, polyA, BssHII, SphI, HincII, XhoI, AvaI, SphI, XbaI, BclI

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XhoI
AscI
BssHII
NdeI
ClaI
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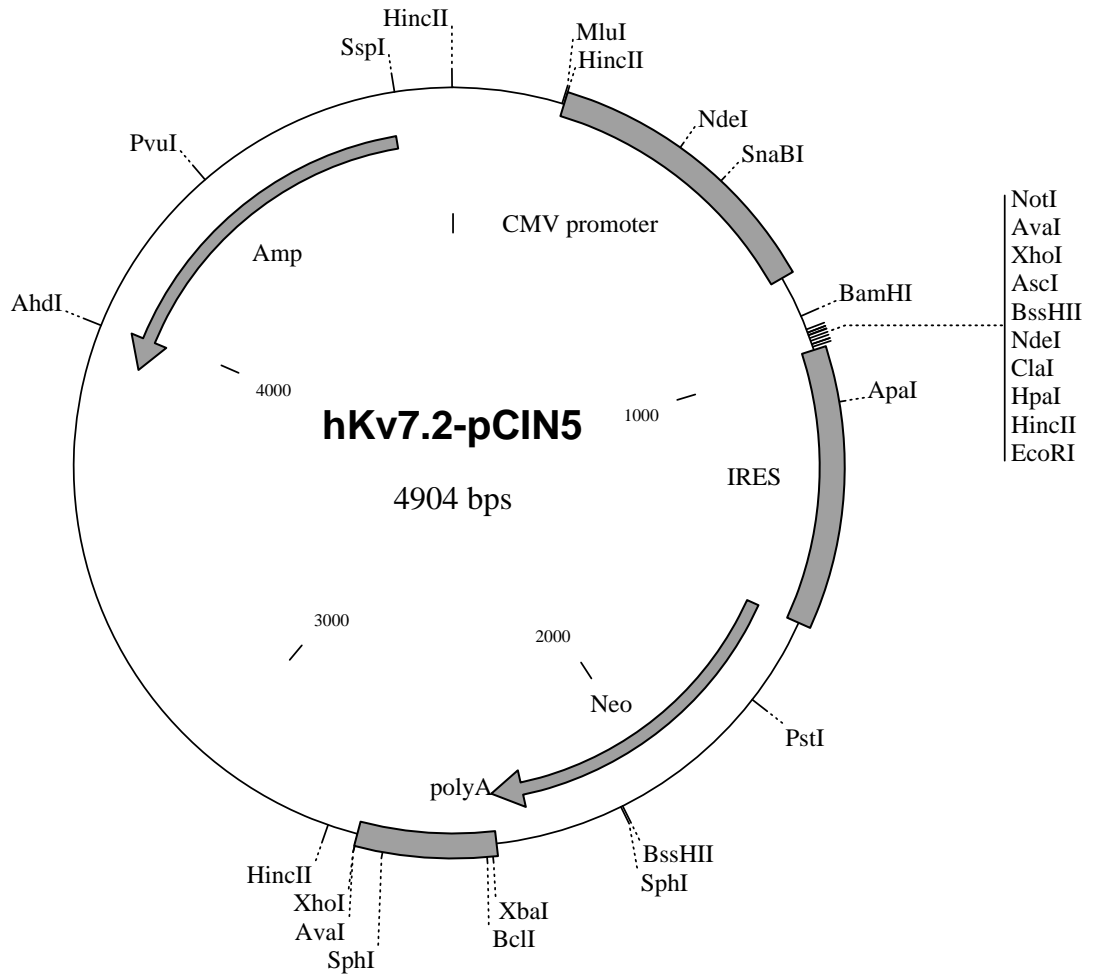
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hKv7.3-pCIH

5726 bps
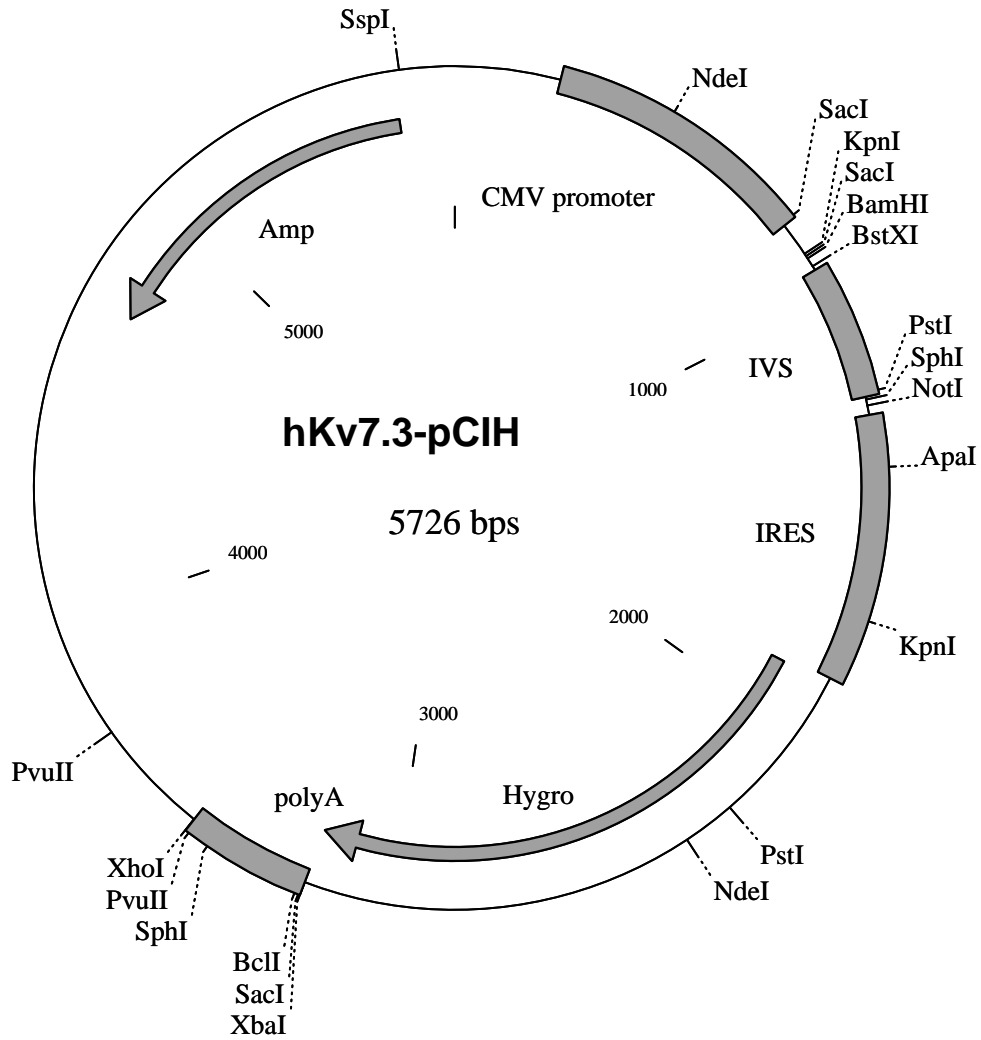
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hKv7.2 Sequence:

The sequence of hKv7.2 used to create this stable cell line contains the following 2 silent base changes with respect the GenBank accession number Y15065.

Bases 2254-2256:   ACC-ACG (Thr - silent)
Bases 2380-2382:   GCG-GCT (Ala - silent)

```
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hKv7.3 Sequence (Accession Number NM_004519):

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References:

Adams, P. R., Brown, D. A., and Constanti, A. (1982) M-currents and other potassium currents in bullfrog sympathetic neurones *J Physiol* **330**, 537-572.

Brown, D. A., and Adams, P. R. (1980) Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate neurone *Nature* **283**(5748), 673-676.

Chung, H. J., Jan, Y. N., and Jan, L. Y. (2006) Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-terminal domains *Proc Natl Acad Sci U S A* **103**(23), 8870-8875.

Cooper, E. C. (2001) Potassium channels: how genetic studies of epileptic syndromes open paths to new therapeutic targets and drugs *Epilepsia* **42 Suppl 5**, 49-54.

Costa, A. M., and Brown, B. S. (1997) Inhibition of M-current in cultured rat superior cervical ganglia by linopirdine: mechanism of action studies *Neuropharmacology* **36**(11-12), 1747-1753.

Guo, J., and Schofield, G. G. (2002) Histamine inhibits KCNQ2/KCNQ3 channel current via recombinant histamine H(1) receptors *Neurosci Lett* **328**(3), 285-288.

Hadley, J. K., Noda, M., Selyanko, A. A., Wood, I. C., Abogadie, F. C., and Brown, D. A. (2000) Differential tetraethylammonium sensitivity of KCNQ1-4 potassium channels *Br J Pharmacol* **129**(3), 413-415.

Jentsch, T. J. (2000) Neuronal KCNQ potassium channels: physiology and role in disease *Nature reviews* **1**(1), 21-30.

Lamas, J. A., Selyanko, A. A., and Brown, D. A. (1997) Effects of a cognition-enhancer, linopirdine (DuP 996), on M-type potassium currents (IK(M)) and some other voltage- and ligand-gated membrane currents in rat sympathetic neurons *Eur J Neurosci* **9**(3), 605-616.

Main, M. J., Cryan, J. E., Dupere, J. R., Cox, B., Clare, J. J., and Burbidge, S. A. (2000) Modulation of KCNQ2/3 potassium channels by the novel anticonvulsant retigabine *Molecular pharmacology* **58**(2), 253-262.

Passmore, G. M., Selyanko, A. A., Mistry, M., Al-Qatari, M., Marsh, S. J., Matthews, E. A., Dickenson, A. H., Brown, T. A., Burbidge, S. A., Main, M., and Brown, D. A. (2003) KCNQ/M currents in sensory neurons: significance for pain therapy *J Neurosci* **23**(18), 7227-7236.

Wang, H. S., Pan, Z., Shi, W., Brown, B. S., Wymore, R. S., Cohen, I. S., Dixon, J. E., and McKinnon, D. (1998) KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel *Science* **282**(5395), 1890-1893.

Zhang, H., Craciun, L. C., Mirshahi, T., Rohacs, T., Lopes, C. M., Jin, T., and Logothetis, D. E. (2003) PIP(2) activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents *Neuron* **37**(6), 963-975.