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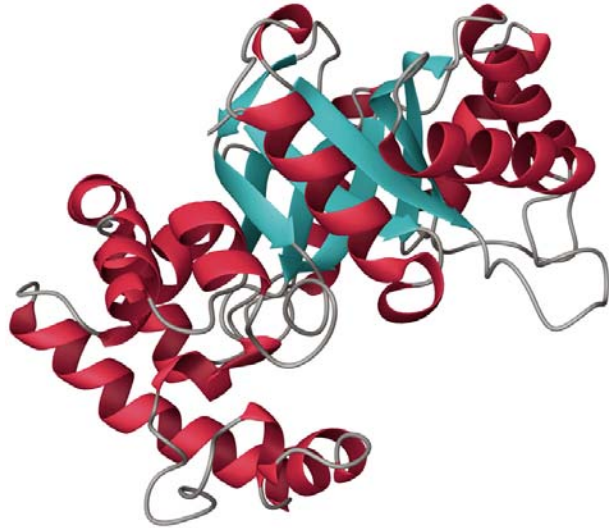
hKv3.2-CHO K1 Recombinant Cell Line

cat. #CYL3044

Revision 1



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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.

Use of IRES is covered by U.S. Patent 4,937,190 and is limited to use solely for research purposes. Any other use of IRES requires a license from Wisconsin Alumni Research Fund (WARF).

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.

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Product description:
Recombinant CHO-K1 cell line expressing the human potassium channel Kv3.2.

Format:
2 x 1ml vials (2.64 x 10^6 cells/ml) of hKv3.2-CHO-K1 at passage 7.

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

Functional Validation:

CHO-K1 cells expressing hKv3.2 were characterised in terms of their pharmacological and biophysical properties using whole-cell patch clamp techniques and IonWorks™ HT.

Using whole-cell patch clamp techniques the threshold of current activation was found to occur when the membrane potential was depolarised to potentials more positive than -30 mV. The mean current at +40 mV was 9.55 ± 4.10 nA (n=7).

Currents were inhibited by the known potassium channel blockers TEA and 4-AP in sub and low mM concentrations.

The currents obtained with IonWorks™ HT were typical of homomeric hKv3.2 currents since they had an initial fast transient component followed by a sustained component.

Functional channel expression over time was monitored using IonWorks™ HT. Channel expression is robust over at least 30 passages. At passage 30, 88% of cells sealed with a resistance >50 MOhm. Of these, 100% expressed hKv3.2 currents >500 pA with a mean current amplitude of 6.80 ± 0.12 nA (n=169).

IonWorks™ HT is a trademark of Molecular Devices Corporation

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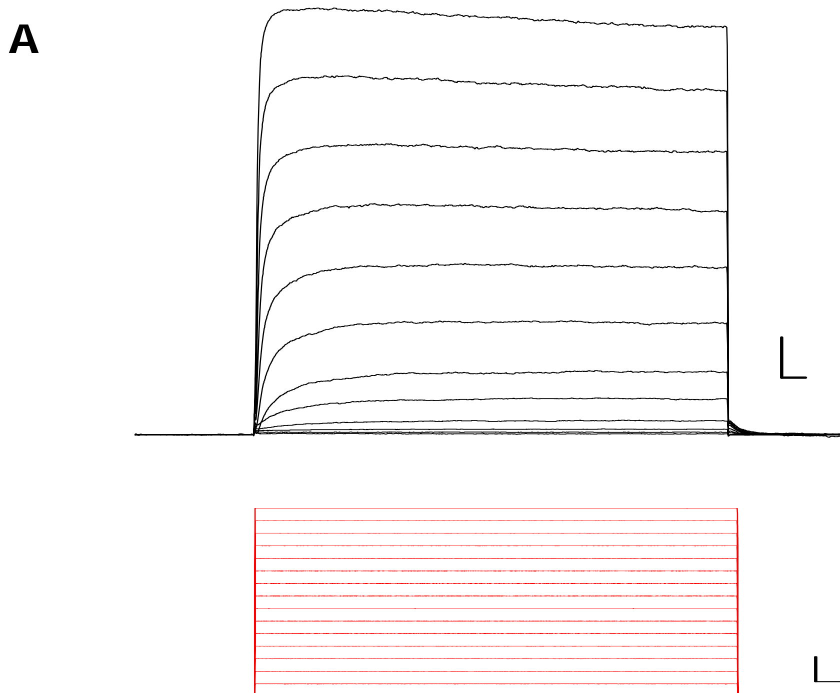
Electrophysiological Properties of the hKv3.2 current.

Conventional Whole-Cell Patch Clamp Electrophysiology.

Current/Voltage Relationship:
The Kv3.2 channel is a very slow inactivating delayed rectifying voltage-gated potassium channel that is found mainly in the brain (Rudy & McBain, 2001; Gutman *et al.*, 2005).
The biophysical properties of the hKv3.2 current were studied by stepping from the holding potential of the cell (-80 mV) to voltages of −70 mV to +40 mV in 10 mV increments every 10 s. The evoked outward currents showed very little inactivation (**Figure 1A**). Substantial currents were only apparent when the membrane potential was depolarised to potentials more positive than -30 mV as expected if the current was carried by hKv3.2 channels (**Figure 1B**). Interestingly, the currents recorded under these conditions do not show the initial fast inactivating phase ascribed to potassium accumulation as seen in the IonWorks™ HT experiments (**Figure 2**). This may be due to the larger bath volume and continuous perfusion used in the conventional patch experiments.

Figure 1. Current/Voltage relationship.
A. Typical current traces (upper panel) elicited by 200 ms depolarising voltage pulses from −70 mV to +40 mV in 10 mV increments (lower panel) from a holding potential of −80 mV. Scale bars represent 10 ms and 1 nA (upper panel) and 10 ms and 20 mV (lower panel) respectively.
B. Mean I/V relationship. Current amplitudes were measured at the end of the 200 ms step and plotted against the test voltage. Currents were normalized to the current amplitude obtained at +40 mV. The mean current at +40 mV was 9.55 ± 4.10 nA (n=7).



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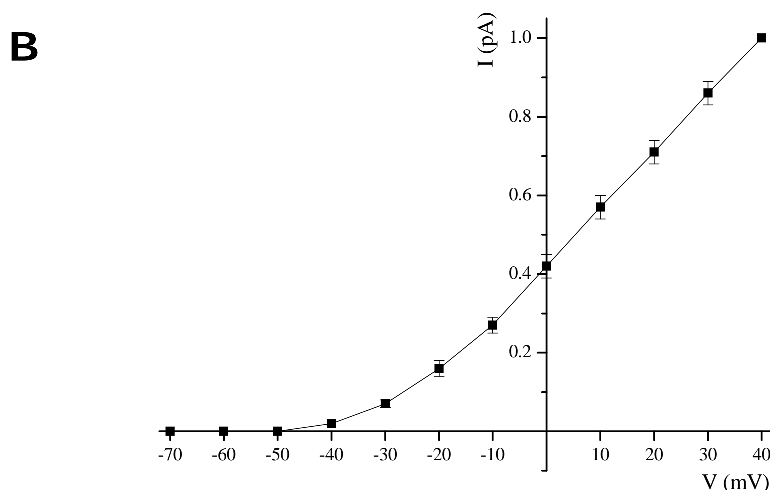
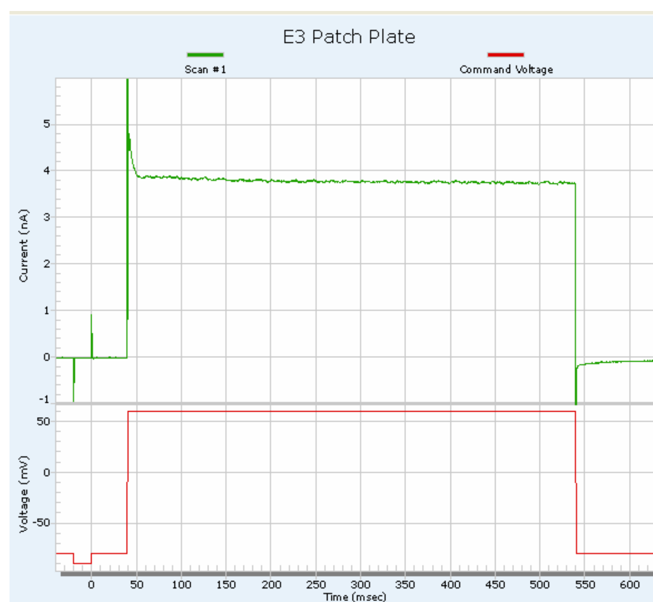


IonWorks™ HT Electrophysiology.

To activate the currents the membrane voltage was stepped from a holding potential of -80 mV to +60 mV for 500 ms before returning to the holding potential. A typical current evoked by this protocol is shown in **Figure 2**. This current trace shows all the hallmarks of homomeric hKv3.2 currents since it has an initial fast transient component, thought to be due to local potassium accumulation resulting from fast activation (Lewis *et al.*, 2004), followed by a sustained component, indicative of negligible inactivation over the duration of the 500 ms test pulse (Hernandez-Pineda *et al.*, 1999).

Figure 2. A typical evoked current on IonWorks™ HT. A typical current (green trace) evoked by a voltage step to +60 mV (red trace).



Manual Patch Clamp Electrophysiology.

Pharmacology – 4-Aminopyridine:

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The cell line evoked currents that were dose-dependently inhibited by 4-Aminopyridine (4-AP), an effect that was rapidly reversible (**Figure 3**). The mean dose-response data is shown in **Table 1**. The cell line is clearly sensitive to sub-mM concentrations of 4-AP as expected for the selective expression of hKv3.2.

Figure 3. The effect of 4-AP on hKv3.2 currents.
A. Current traces evoked by stepping to −10 mV from a holding potential of −80 mV in the presence of various concentrations of 4-AP. Upper panel: before the addition of 4-AP (control - black trace), after addition of 0.5 mM 4-AP (green), 1 mM 4-AP (blue) and 5 mM 4-AP (red) respectively. Scale bars represent 100 ms (x-axis) and 500 pA (y-axis). Lower panel: voltage-step protocol. Scale bars represent 100 ms (x-axis) and 35 mV (y-axis).
B. Mean currents evoked by stepping to −10 mV for 1 s from −80 mV. Currents normalized to control current. hKv3.2 channels were stepped for 1 s every 12 s.

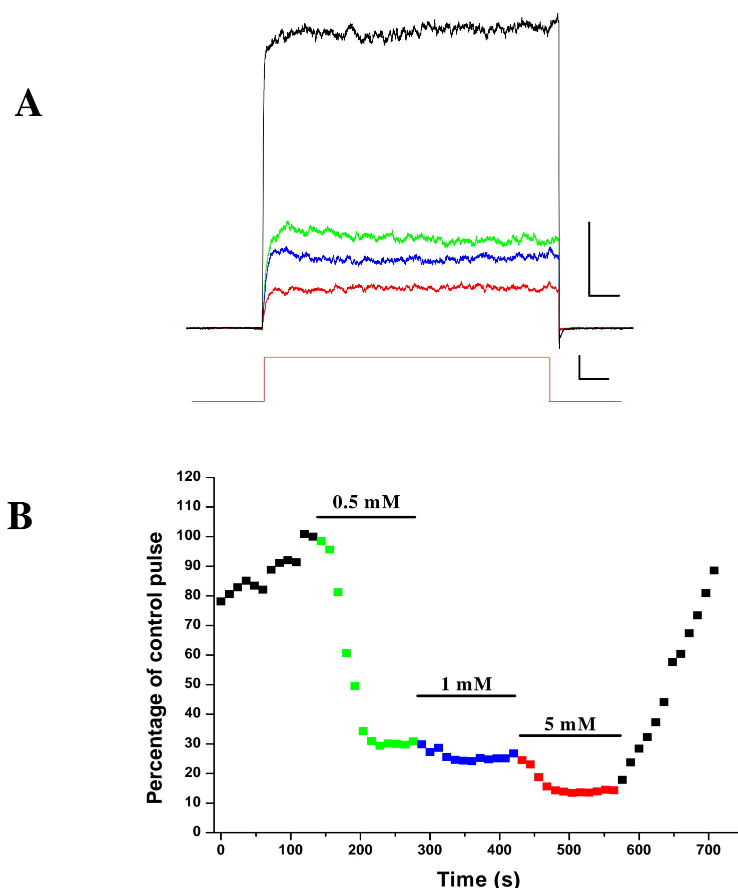

Table 1. Mean 4-AP dose-response data. Percentage of control current remaining following 3 minutes of application of different concentrations of 4-AP (n=4-6).

Concentration of 4-AP (mM)	Percentage inhibition of control current (mean ± SEM)
0.5	69.6 ± 2.0
1	73.3 ± 3.1
5	85.7 ± 1.1

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Pharmacology – Tetraethylammonium chloride:
Tetraethylammonium chloride (TEA) also blocked hKv3.2 currents as expected (**Figure 4**) with an estimated IC_{50} of around 1 mM (**Table 2**).

Figure 4. The effect of TEA on hKv3.2 currents.
A. Current traces evoked by stepping to -10 mV from a holding potential of –80 mV in the presence of various concentrations of TEA. Upper panel: before the addition of TEA (control - black trace), after addition of 1 mM TEA (blue), 5 mM TEA (green), 10 mM TEA (pink) and after wash off of TEA (red). Scale bars represent 100 ms (x-axis) and 1 nA (y-axis). Lower panel: Voltage-step protocol. Scale bars represent 100 ms (x-axis) and 35 mV (y-axis).
B. Typical hKv3.2 currents produced by depolarising pulse in the presence of various concentrations of TEA. hKv3.2 channels were stepped from a holding potential of –80 mV to 0 mV for 1 s every 12 s.

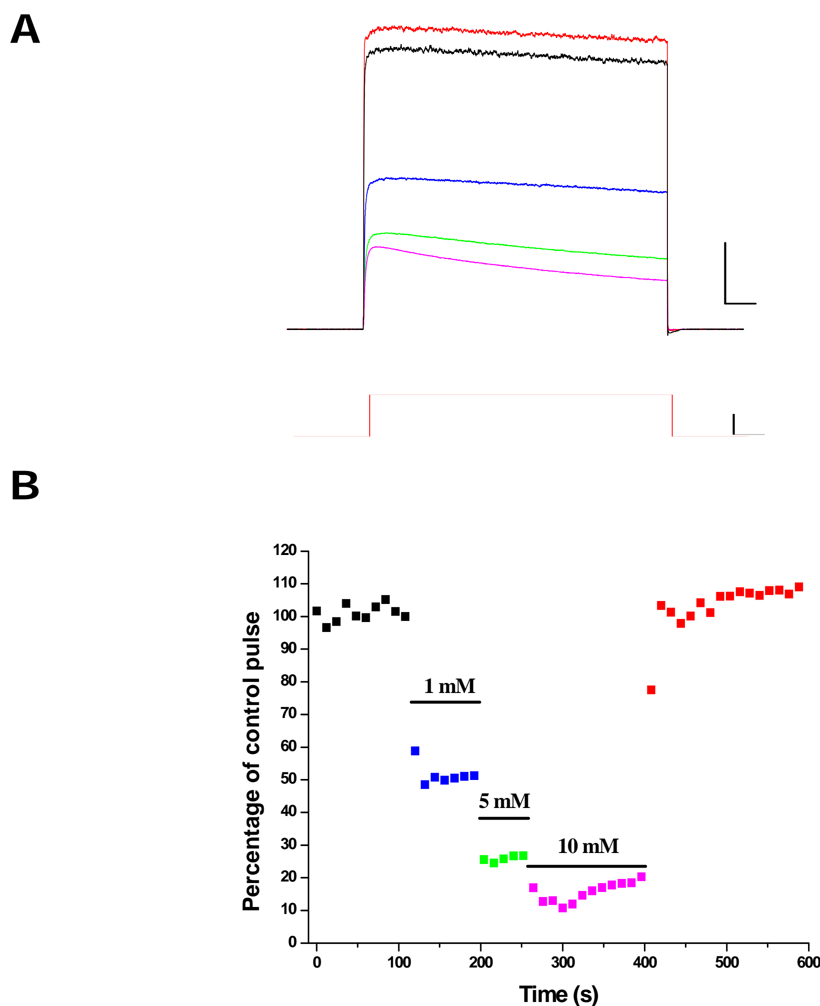

Table 2. Mean TEA dose-response data. Percentage of control current remaining after application of different concentrations of TEA (n=3).

Concentration of TEA (mM)	Percentage inhibition of control current (mean ± SEM)
1	50.1 ± 2.1
5	79.0 ± 2.9
10	83.6 ± 2.5

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IonWorks™ HT Electrophysiology.

Pharmacology – 4-AP and TEA:
The effect of 4-AP (**Figure 5**) and TEA (**Figure 6**) was tested using a 4 s voltage test step to +60 mV. The hKv3.2-CHO K1 cell line was sensitive to low mM concentrations of both 4-AP (IC_{50} values were 6 mM and 3 mM for 4-AP and TEA respectively). The data generated on IonWorks™ HT is in agreement with previous literature reports. The Kv3.2 channel is reported to be highly sensitive to both 4-AP and TEA with concentrations of less than 1 mM reducing the current produced by approximately 50% (Coetzee *et al.*, 1999; Hernandez-Pineda *et al.*, 1999; Lien *et al.*, 2002).

Figure 5. The effect of 4-AP on hKv3.2 currents.

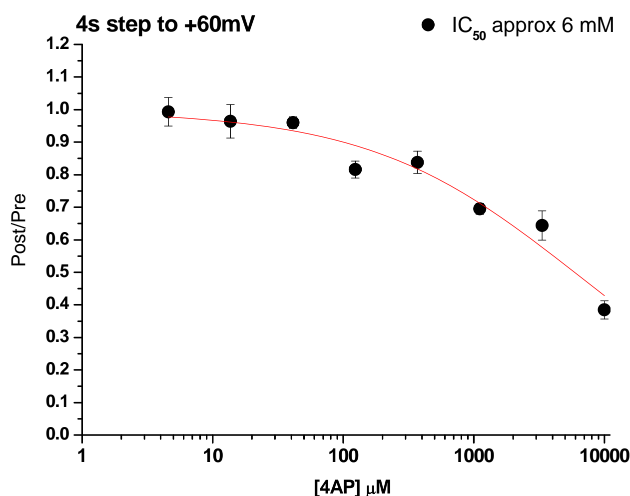
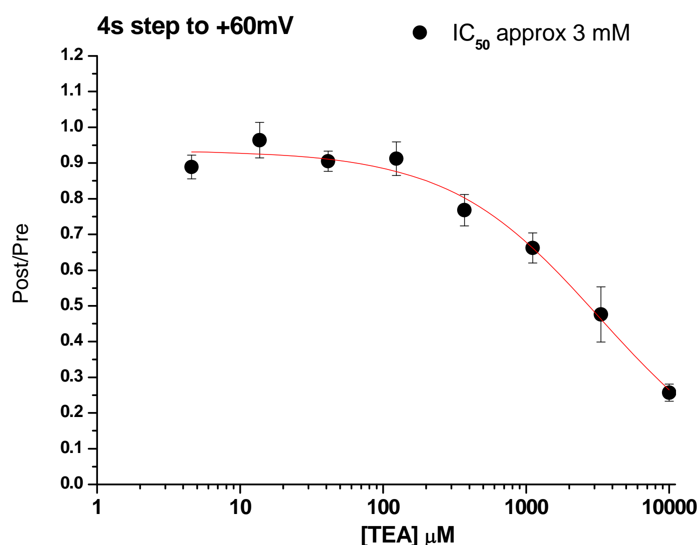


Figure 6. The effect of TEA on hKv3.2 currents.



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Stability of hKv3.2-CHO K1 Cell Line.

IonWorks™ HT Electrophysiology.

The hKv3.2-CHO K1 cell line has stable expression for > 30 passages.

Functional channel expression, defined as cells expressing hKv3.2 current of ≥ 500 pA, was monitored using IonWorks™ HT. This data and the mean current amplitude is shown in **Figure 7**. Number of cells expressing a current ≥ 500 pA shown above mean current amplitude data. Sealing data is shown in **Figure 8**.

Figure 7. Stability of expression over passage.
The upper panel shows the percentage of cells expressing a mean peak tail current >500 pA at cell passages 1, 6, 19, 24, and 30. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles).

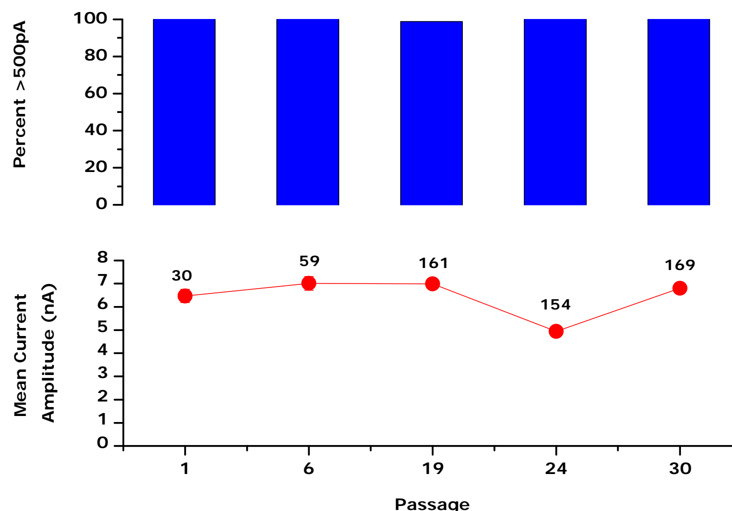
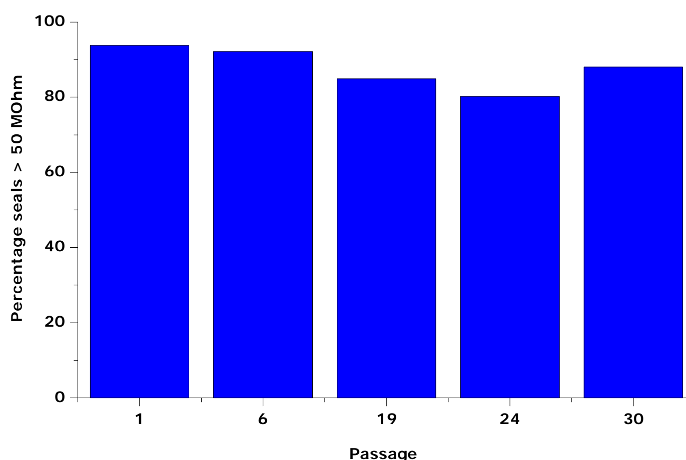


Figure 8. Sealing rates over passage.
The percentage of cells sealing (defined as a seal resistance of >50 MOhm).



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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) + Glutamax medium supplemented with 10% Foetal Bovine Serum plus 400 µg/ml of Geneticin to ensure that the recombinant expression is maintained.

Transfection of CHO-K1 host cells with the human Kv3.2-pCIN5 construct does not appear to have retarded the growth characteristic of the host cells, which exhibit 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 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. 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 (400 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™ I)	(Invitrogen	#31765)
10% Foetal Bovine Serum	(Invitrogen	#16000)
400 µg/ml Geneticin	(Invitrogen	#10131)

Other reagents required:

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

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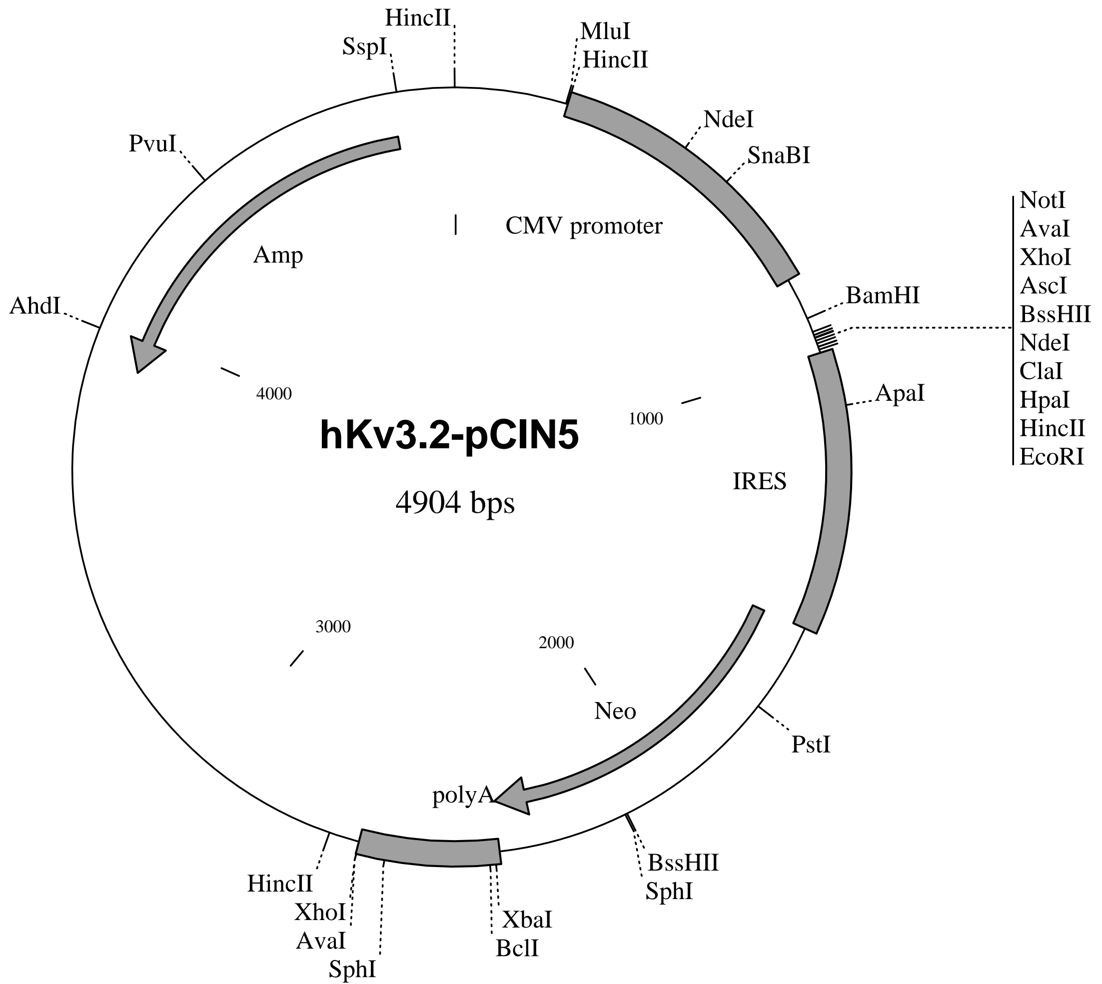
Vector:



Polylinker: CMV-BamHI-NotI-AvaI-XhoI-AscI-**hKv3.2**-HpaI-EcoRI-IRES-*neo*

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hKv3.2 Sequence:

The sequence of the cDNA used to make this cell line contains one silent mutation with respect to the accession number (NM_139136) AC**A**-AC**G** (Thr) - at position 1535.

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