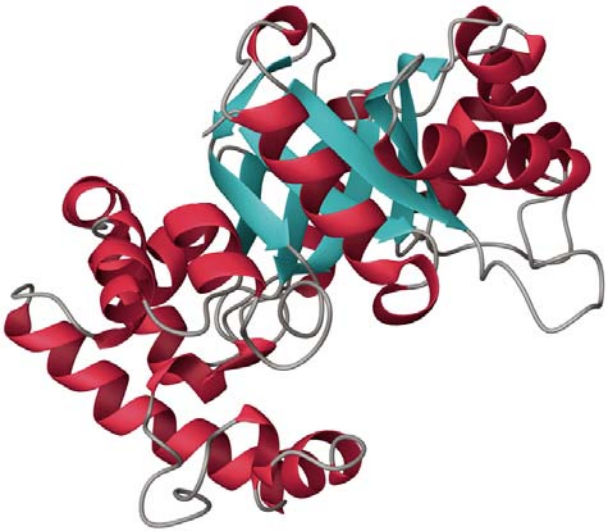
MILLIPORE

hKv2.1/hKv9.2-CHO K1 Recombinant Cell Line

cat. #CYL3067

Revision 1



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Contents:

Licensing Statement

Product description:
Recombinant HEK293 cell line coexpressing the human Kv2.1 [voltage-gated potassium channel, Shab-related subfamily, member 1 (KCNB1) – Accession Number NM_004975] and human Kv9.2 (voltage-gated, delayed-rectifier potassium channel - Accession Number NM_020697).

Format:
2 x 1 ml aliquots containing 1.41 x 10^6 cells/ml in 7.5% DMSO at passage 13.

Mycoplasma Testing:
The cell line has been screened using the MycoSensor™ PCR Assay Kit (Stratagene) to confirm the absence of Mycoplasma species.

Functional Validation:

CHO-K1 cells expressing hKv2.1/hKv9.2 were characterised in terms of their biophysical and pharmacological properties using whole-cell patch clamp techniques. The mean current amplitude at +60 mV was 4.9 ± 0.8 nA (n = 9) for hKv2.1/hKv9.2 (8.4 ± 3.1 nA (n = 6) for hKv2.1 when expressed alone).

The threshold for current activation was between -20 and -30 mV. This property is characteristic of both hKv2.1 and hKv2.1/hKv9.2 currents described in the literature.

The cell-line is shown to be expressing hKv2.1 current since it was inhibited by low mM concentrations of the potassium channel blocker tetraethylammonium (5 mM; 56.7 ± 1.2% inhibition, n = 7) which is similar to hKv2.1 when expressed alone (5 mM; 63.3 ± 1.1%, n = 4). The sensitivity of hKv2.1/hKv9.2 to low mM concentrations of the potassium channel blocker 4-aminopyridine was reduced (5 mM; 15.4 ± 2.3%, n=6) compared to that of hKv2.1 when expressed alone (1 mM; 33.8 ± 1.5%, n = 3). This is in agreement with published findings that have shown that the sensitivity of Kv2.1 to 4-AP is greatly reduced (10-fold) when co-expressed with a Kv9.x subunit (Patel *et al.*, 1997).

Functional channel expression over time was monitored using IonWorks™ HT. Channel expression is robust over at least 35 passages: 95% of cells expressed outward current >500 pA at passage 35 (n=98). The mean current amplitude of these cells was 1.44 nA. 64% of cells had a seal resistance of >50 MOhm.

IonWorks™ HT is a trademark of Molecular Devices Corporation

Electrophysiological Properties of the hKv2.1/hKv9.2 Current.

Conventional Whole-Cell Patch Clamp Electrophysiology:

The Kv2.1 channel is a delayed rectifying, non-inactivating voltage-gated potassium channel that is widely expressed in the brain (Trimmer, 1991; Drewe *et al.*, 1992; Gutman *et al.*, 2005), in the heart and in muscle fibres (Gutman *et al.*, 2005). It has been suggested to be critical for maintaining cell membrane potentials and for controlling the excitability of neurons (Misonou *et al.*, 2005).

Kv9.2 is a neuronal subunit that has no functional activity when expressed alone but modulates Kv2.x alpha subunits. Expression of Kv9.2 mRNA has been seen in (mouse) brain only, often expressed with Kv2.1 (Salinas *et al.*, 1997). The highest expression levels were in the main olfactory bulb, cerebral cortex, hippocampal formation, habenula, basolateral amygdaloid nuclei, cerebellum and also in spinal cord. No expression was observed in heart, spleen, lung, liver, skeletal muscle, kidney, or testis.

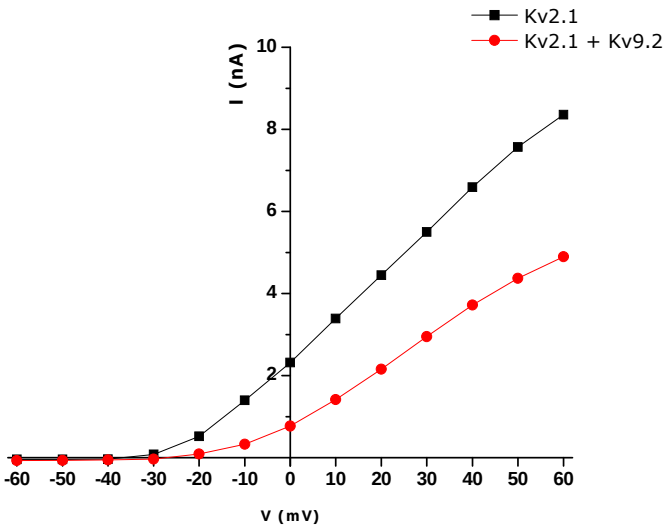
From the literature it appears that there are 3 characteristic electrophysiological features that distinguish Kv2.1/Kv9.2 expression from that of Kv2.1 alone (Salinas *et al.*, 1997). Firstly there should be a reduction in current amplitude at all voltages with Kv9.2 expression. Secondly there is likely to be a deviation from linearity in the I/V relationship beyond +50 mV with co-expression of Kv9.2. Finally there is a shift in the steady state inactivation properties.

Current/Voltage Relationship:

Current-voltage (I/V) relationship of hKv2.1 and hKv2.1/hKv9.2:

The I/V relationship for both cell lines is shown in **Figure 1.** The biophysical properties of the hKv2.1 and hKv2.1/hKv9.2 channels were studied by stepping from the holding potential of the cell (-80 mV) to voltages of –60 mV to +60 mV in 10 mV steps every 10 s. The duration of each step was 200 ms.

Figure 1. I/V relationship in hKv2.1- and hKv2.1/hKv9.2-CHO K1 cell lines.

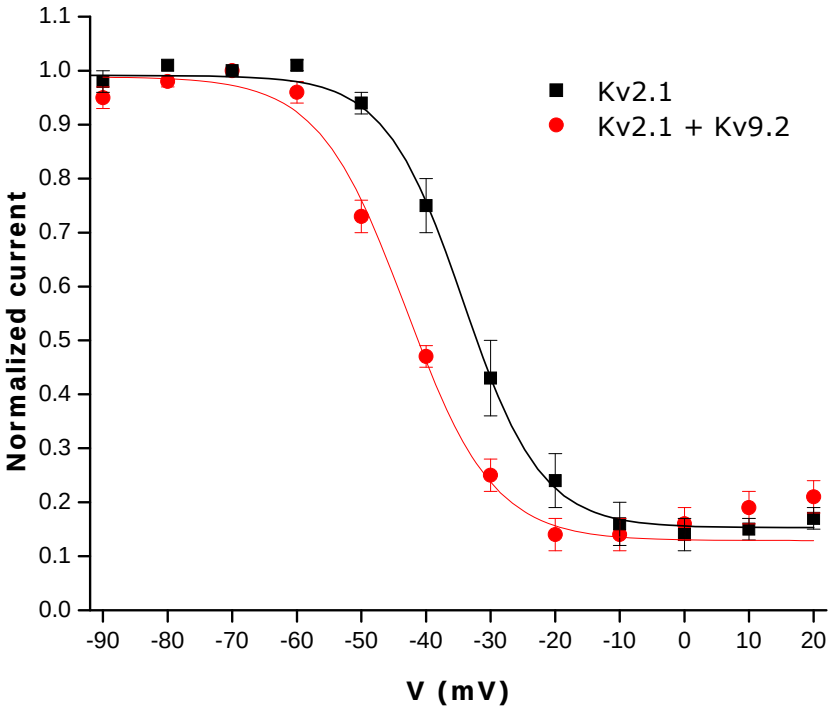


Currents from both cell lines activated at membrane potentials between -20 and -30 mV. This is in agreement with previously published findings for Kv2.1 expressed alone (Shi *et al.*, 1994; Wible *et al.*, 1997; Coetzee *et al.*, 1999) and for the co-expression of Kv2.1 and Kv9.2 (Salinas *et al*., 1997) . Mean currents at +60 mV were 8.4 ± 3.1 nA (n = 6) and 4.9 ± 0.8 nA (n = 9) for hKv2.1 and hKv2.1/hKv9.2 respectively. The reduction in the size of the potassium currents elicited at all membrane potentials without modifying the activation threshold is comparable to the findings of Salinas *et al*., 1997. Here, on pulsing to +30 mV, an approximate 45% reduction of the Kv2.1 current was observed in *Xenopus* oocytes co-injected with mouse RNA of both subunits (1:1 ratio) in comparison to oocytes injected with Kv2.1 RNA only. This confirms the modulatory presence of the Kv9.2 subunit.

It can also be noted from the I/V relationship (**Figure 1**) that when expressed alone the hKv2.1 channel displays outward currents with very little inactivation, whereas the hKv2.1/hKv9.2 current deviates from linearity at voltages more positive than +40/+50 mV, again in agreement with Salinas *et al*., 1997.

Inactivation:
The $V_{½}$ inactivation was found by measuring the peak current at test voltage of +30 mV for 200 ms after pre-pulses ranging from −90 to +20 mV in 10 mV steps for 10 s. Cells held at −80 mV and sweeps were every 20 s. The $V_{½}$ of inactivation for hKv2.1 alone was found to be approximately −34.3 ± 1.7 mV, with a slope (k) of 6.1 ± 0.3 (n = 3; **Figure 2**). In cells expressing hKv2.1/hKv9.2 the $V_{½}$ value was shifted almost 10 mV to more negative voltages ($V_{½}$ = -43.3 ± 1.0 mV, k = 6.7 ± 0.2, n = 5). This degree of shift of $V_{½}$ is in line with the results of Salinas *et al*., 1997 (Kv2.1: $V_{½}$ of inactivation = -20.5 ± 0.9 mV and Kv2.1/Kv9.2: -35.7 ± 3.2 mV, NB these data were with mouse homologue) and is further confirmation of the expression of the hKv9.2 subunit.

Figure 2. Inactivation of the hKv2.1/hKv9.2 current.
Cells were stepped from a holding potential of −80 mV to a test pulse of 30 mV for 200 ms following pre-pulse voltages ranging from -90 to +20 mV for 10 s in 10 mV steps. Sweeps every 20 s. Currents normalized to current at pre-pulse of −70 mV. n = 3 for hKv2.1 and n = 5 for hKv2.1/hKv9.2.



Pharmacology:

It has been reported that Kv2.1 currents are blocked by tetraethylammonium chloride (TEA) and 4 aminopyridine (4-AP) with IC_{50} values in the low mM range (Bowden *et al.*, 2003). The sensitivity of Kv2.1 to 4-AP has been shown to be reduced upon co-expression with Kv9.x (Patel *et al.*, 1997). Here we show the effect of application of 5 mM concentrations of both compounds on the potassium current. 5 mM TEA (**Figure 3**) and 4-AP (**Figure 4)** block the steady state hKv2.1 current by approximately 57% and 15% respectively.

Pharmacology – TEA:

Figure 3. Effect of 5 mM TEA on hKv2.1/hKv9.2 currents.
A. Current traces evoked by stepping to +40 mV for 1 s from a holding potential of –80 mV, before (black trace) and after application of (red) 5 mM TEA. Traces show the level of steady state block that is achieved after an approximate 80 s incubation with 5 mM TEA. Scale bars represent 200 ms and 1 nA.
B. Typical time course of inhibition of hKv2.1/hKv9.2 currents by 5 mM TEA. Currents were evoked by stepping to +40 mV for 1 s from -80 mV. Sweeps every 10 s. Currents normalized to current before the addition of TEA.

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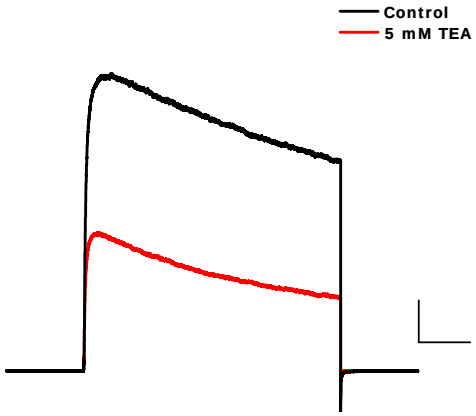

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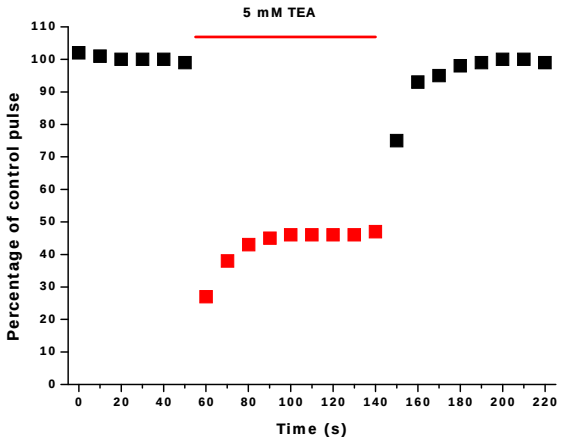

Pharmacology – 4-AP:

Figure 4. Effect of 5 mM 4-AP on hKv2.1/hKv9.2 currents.
A. Current traces evoked by stepping to 40 mV for 1 s from a holding potential of –80 mV, before (black trace) and after application of (blue) 5 mM 4-AP. Traces show the level of steady state block that is achieved after an approximate 80 s incubation with 5 mM 4-AP. Scale bars represent 200 ms and 500 pA.
B. Typical time course of inhibition of hKv2.1/hKv9.2 currents by 5 mM 4-AP. Currents were evoked by stepping to 40 mV for 1 s from -80 mV. Sweeps every 10s. Currents normalized to current before the addition of 4-AP.

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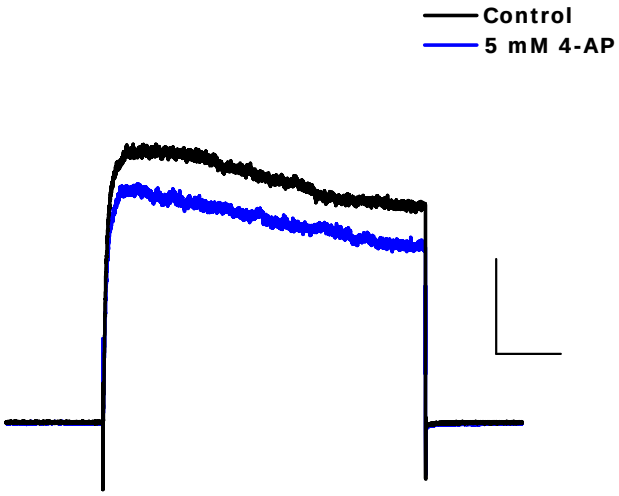

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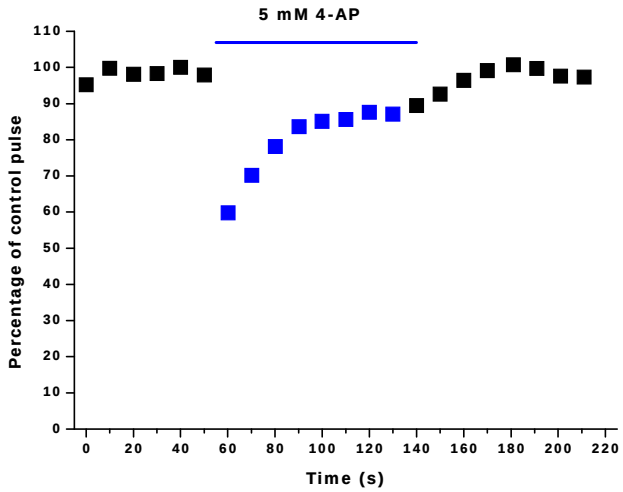


Stability of hKv2.1/hKv9.2-CHO K1 Cell Line.

IonWorks™ HT Electrophysiology.

The hKv2.1/hKv9.2-CHO K1 cell line has stable expression for >35 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 5**. Sealing data is shown in **Figure 6.**

Figure 5. Stability of expression over passage.
The upper panel shows the percentage of cells expressing a mean peak current >500 pA at 40 mV at cell passages 9, 12, 19, 23, 29 and 35. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of cells (numbers above red circles - out of 32 cells for passages 9 & 12 and out of 160 cells for all other passages).

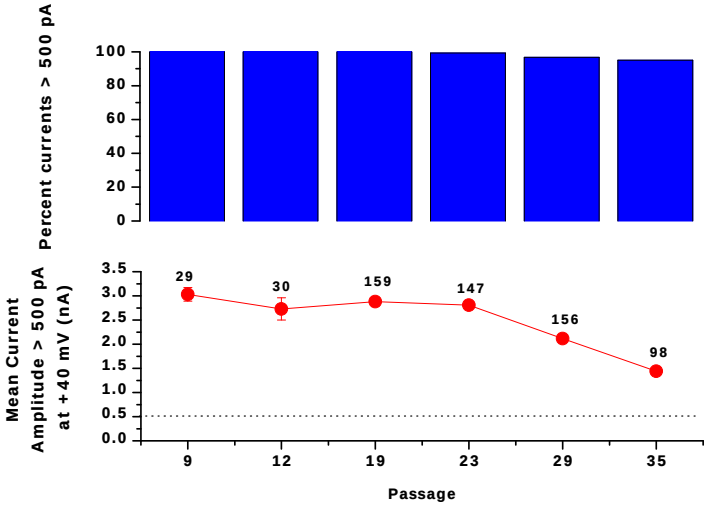
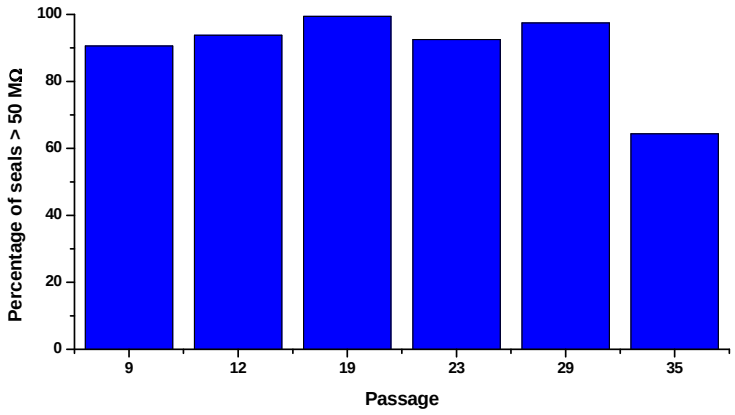
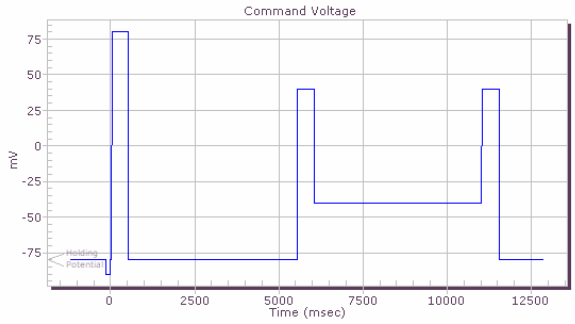


Figure 6. Sealing rates over passage.
The percentage of cells sealing (defined by a seal resistance of >50 MΩ).



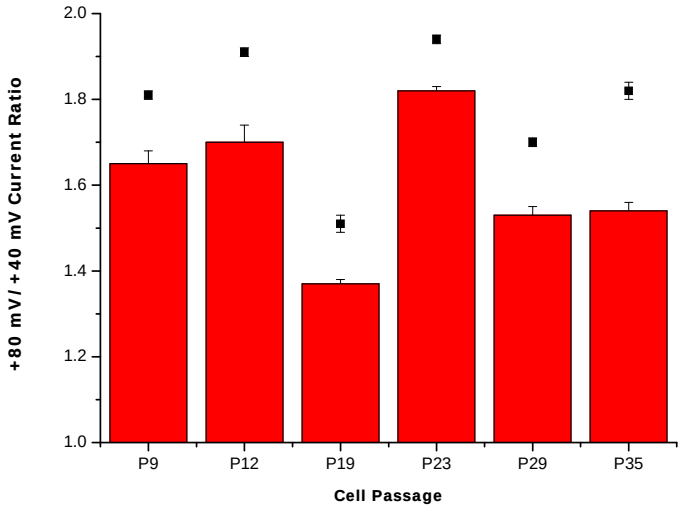
The stable expression of potassium currents in the hKv2.1/hKv9.2-CHO K1 cell line indicates the presence of the hKv2.1 channel. In order to show stable expression of the hKv9.2 subunit the +80 mV/+40 mV current ratio (as determined using the IonWorks™ HT voltage protocol in **Figure 7**) was also monitored over time.

Figure 7. Voltage Protocol. After an initial conditioning prepulse to –90 mV for 100 ms, the voltage was stepped from the holding potential (V_h) of –80 mV to +80 mV for 500 ms before returning back to V_h for a 5 s. The voltage was then stepped up to +40 mV for 500 ms, down to –60 mV for 5 s, and back up to +40 mV for a second 500 ms pulse, before finally returning to V_h.



It was seen that for cells expressing hKv2.1 alone, the current amplitude at the +80 mV step approached twice (1.5 to 1.9-fold) the amplitude that at the subsequent step to +40 mV. However in cells expressing both hKv2.1 and hKv9.2, the current amplitude at the +80 mV step was closer in size to that at the subsequent step to +40 mV (ratios of 1.4-1.8). The +80 mV/+40 mV current ratio in the hKv2.1/hKv9.2-CHO K1 cell line was consistently lower than that in the parental cell line (hKv2.1-CHO K1) in each IonWorks™ HT run, confirming that the expression of hKv9.2 was also stable over this time period (**Figure 8**).

Figure 8. +80 mV/+40 mV current ratios over passage.
Red bars - hKv2.1/hKv9.2-CHO K1, black scatter plots - hKv2.1-CHO K1.



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™ 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 hKv2.1-CHO K1 cells with the hKv9.2 ion channel 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 T75 cm^2 flask containing 20 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 20 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 0.5-1×10^6 cells per T75 cm^2 or 1-2×10^6 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™ 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)

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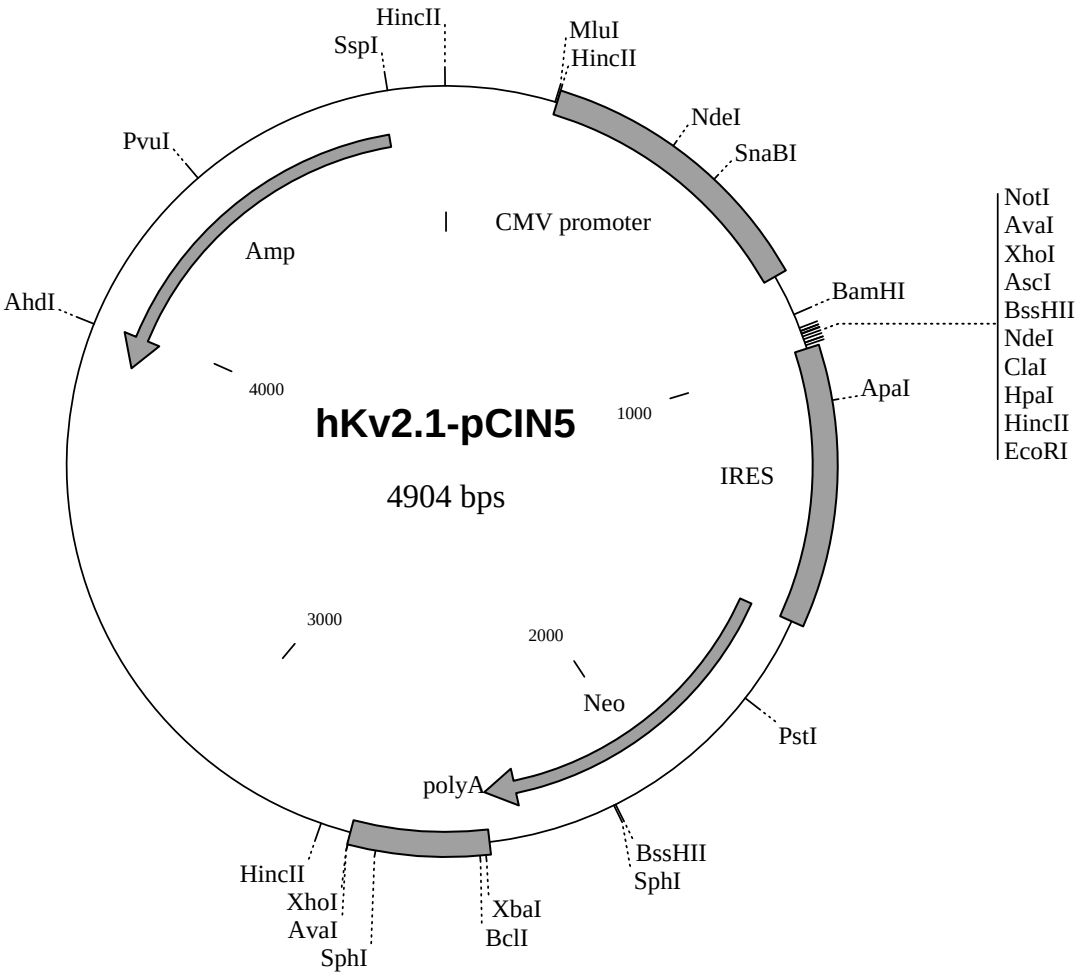

Polylinker: CMV-BamHI-NotI-**hKv2.1**-AscI-ClaI-HpaI-EcoRI-IRES-*neo*

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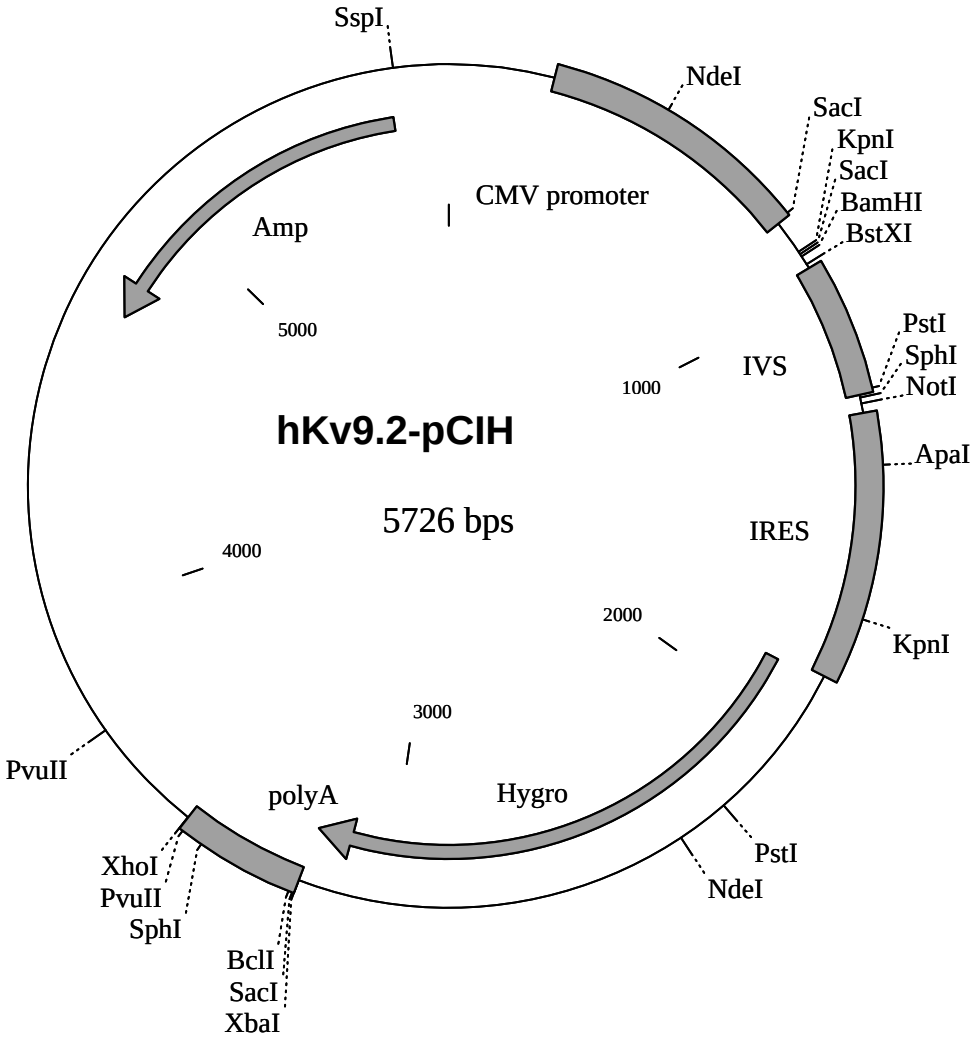

Polylinker: CMV-KpnI-SacI-BamHI-**hKv9.2**-NotI-IRES-hyg

hKv2.1 Sequence (Accession Number NM_004975):

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