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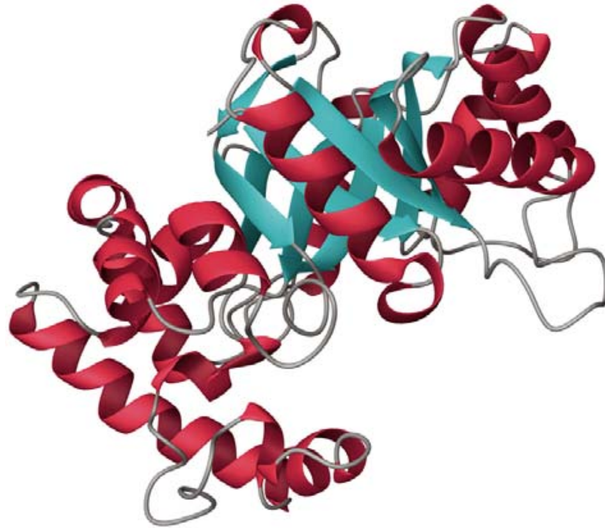
hTRPV1-HEK293 Recombinant Cell Line

cat. #CYL3063

Revision 1



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Contents:

Product description	3
Format	3
Mycoplasma Testing Details	3
Functional Validation Overview	3
Introduction	4
Electrophysiological Properties of the hERG Current	5
Effect of Capsaicin	5
I/V Relationship of the Capsaicin-Evoked Current	6
Pharmacology – Capsazepine	7
Pharmacology - SB366791	8
Media Formulation:	11
Vector:	12
Sequence:	123
References.	14-15

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 HEK293 cell line expressing human TRPV1 [capsaicin receptor / vanilloid receptor (VR1), accession number AF196175].

Format:

2 x 1 ml aliquots containing 3.80 x 10^6 cells/ml in 10% DMSO at passage 13.

Mycoplasma Testing:

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

Functional Validation:

hTRPV1 channels have been selectively expressed in a HEK293 cell line and characterized in terms of their biophysical and pharmacological properties using whole-cell patch clamp techniques.

hTRPV1 currents were activated by capsaicin in the same concentration range as reported previously (0.1-1 µM) and displayed outward rectification with a reversal potential close to 0mV indicative of a non-selective cation current.

hTRPV1 currents evoked by capsaicin were blocked by the competitive antagonist capsazepine (50 µM) and the more selective and potent competitive TRPV1 antagonist SB-366791 in the low nanomolar range.

hTRPV1 current amplitudes, monitored using IonWorks™ HT, were consistently in excess of 2 nA and percent expression levels above 60% (currents > 500 pA) for > 40 cell passages.

These data demonstrate the selective and stable functional expression of TRPV1 channels in this HEK293 cell line.

IonWorks™ HT is a trademark of Molecular Devices Corporation

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Introduction.

The TRPV1 channel belongs to the transient receptor potential channel (TRP) super-family that consists of at least 6 subfamilies. The channels exist as tetramers where each subunit consists of 6 putative trans-membrane domains. In this respect they have a similar topology to Kv channels. The vast majority are permeable to both monovalent cations and calcium (see Clapham *et al*., 2001).

Specifically, TRPV1 channels belong to the Thermo-TRP subfamily and are activated by various endogenous stimuli such as noxious heat, low pH and a number of inflammatory lipid metabolites. They are also activated by the vanilloid capsaicin, the key ingredient in hot chilli peppers that causes a burning sensation when topically applied or ingested (see review of Huang *et al*., 2006). It is through activation of this receptor that these effects are mediated and thus originally termed VR1 (vanilloid receptor 1).

Activation of the receptor has an important role in the sensation of pain caused by noxious agents. Consistent with this role, TRPV1 channels are located both peripherally and centrally on neurons that are known to be involved in the transmission of pain such as certain subsets of nociceptive neurons found in sensory ganglia (Levine and Alessandri-Haber, 2007).

A number of pharmaceutical companies are trying to develop potent TRPV1 antagonists in the hope of preventing pain by blocking this sensor (Gunthorpe *et al*., 2004, Cui *et al*., 2006). This approach has met with some success, with a number of drugs in Phase I/II clinical trials (see review of Szallasi *et al*., 2007). Another strategy has been to develop TRPV1 agonists that work by causing desensitization of sensory neurons albeit after initial stimulation, that can actually cause pain before analgesia ensues.

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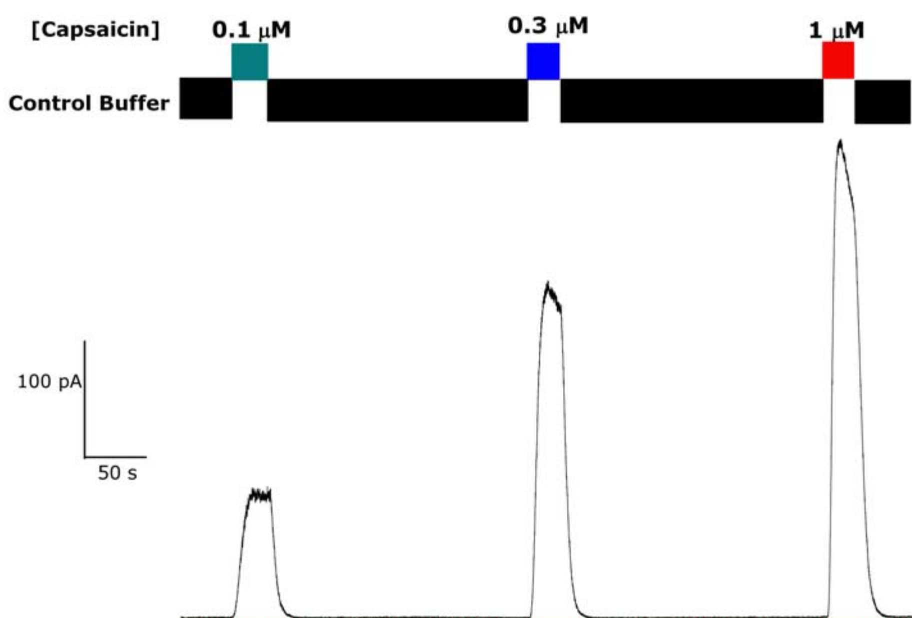
Electrophysiological Properties of the hERG Current.

Conventional Whole-Cell Patch Clamp Electrophysiology.

Effect of Capsaicin:
Activation by capsaicin and other vanilloids is a hallmark feature of TRPV1 and played a fundamental role in the first successful cloning of the receptor from rodent DRG neurons (Caterina *et al.,* 1997). Reported half maximal effective concentrations for this agonist (EC_{50}) in native cells and recombinant systems are typically in the 0.1-1 micromolar range (1 µM, Oh *et al*., 1996; 0.1 µM , Tominaga *et al.,* 1998; 0.6 µM, Hayes *et al.,* 2000). Consistent with these reports bath-applied capsaicin dose-dependently evoked outward currents at a holding potential of +20 mV in a similar concentration range (**Figure 1**). Furthermore the mean peak current amplitude in the presence of 1 µM capsaicin was 3.7 ± 1.7 nA (n=5); an appropriate level of functional expression for patch clamp experiments.

Figure 1. Effect of Capsaicin on TRPV1 currents.
Cells were clamped at a holding potential of +20 mV and perfused with external buffer. Increasing concentrations of capsaicin were briefly pulsed on to the cell and immediately washed off. Zero current level is shown as the red dotted line.



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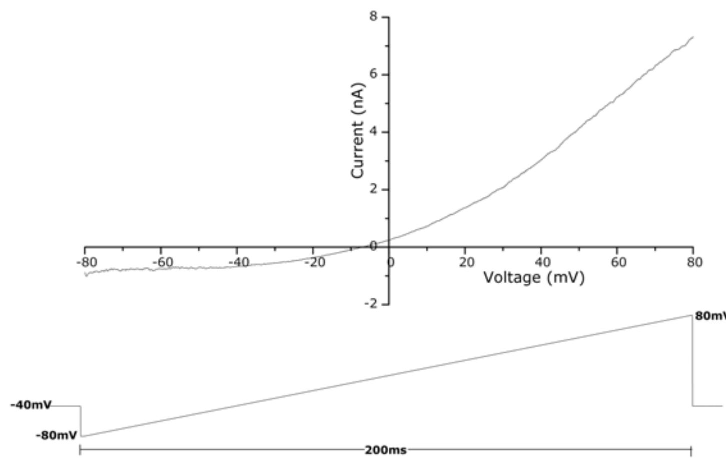
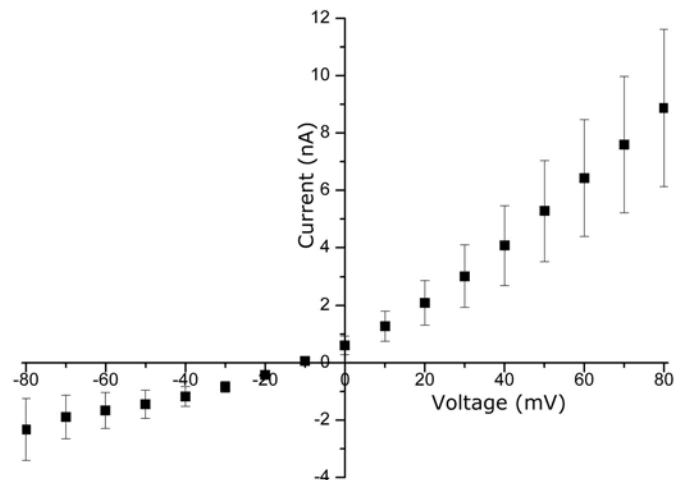
I/V Relationship of the Capsaicin-Evoked Current.

Voltage ramps were applied before and during the plateau phase of the capsaicin (0.3 µM) evoked current. The I/V relationship was obtained by subtracting the I/V relationship prior to capsaicin addition from the relationship obtained in the presence of capsaicin (**Figure 2**). Under these ionic conditions (physiological monovalent cation concentrations and 100 µM external Ca^{2+}) the I/V relationship exhibited outward rectification and reversed slightly negative to 0 mV (-6.6 ± 0.78 mV, n=6) indicative of a non-selective cation current. These features are typical of TRPV1 (Caterina *et al.,* 1997, Oh *et al.*, 1996, Hayes *et al* 2000, McLatchie and Bevan 2001, Gunthorpe *et al.,* 2004).

Figure 2. TRPV1 IV relationship evoked by capsaicin .
A. Cells were held at potential of -40 mV and ramped from –80 mV to –80 mV over 200 ms before and during addition of 0.3 µM capsaicin. The leak-subtracted I/V relationship exhibits outward rectification. See text for further details.



B. The mean I/V relationship for capsaicin evoked currents was obtained by measuring the current evoked at various voltages (–80 mV to +80 mV in 10 mV increments) from individual I/V relationships as shown in **A**.



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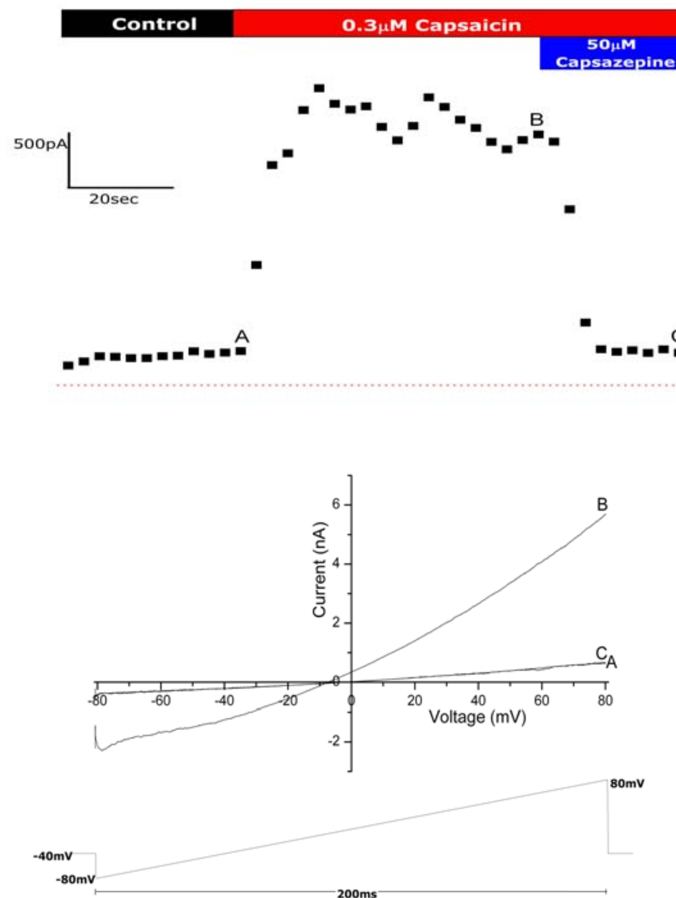
Pharmacology – Capsazepine:
The effect of the TRPV1 competitive antagonist capsazepine (Bevan *et al.,* 1992, Seabrook *et al.*, 2002, Phillips *et al.,* 2004), at a supra-maximal concentration of 50 µM was examined. A typical example is illustrated in the long term record in **Figure 3 (upper panel)** where outward membrane current was recorded at +40 mV every 3 s, prior to any additions (Control, black bar), in the presence of capsaicin (0.3 µM, red bar) and subsequently in the presence of capsaicin and capsazepine (50 µM, blue bar). Capsaicin rapidly evoked outward currents that were completely abolished by capsazepine (99.1 ± 0.81% inhibition, n=3). Individual trace records taken at points A, B and C are shown in **Figure 3 (lower panel)**. Capsaicin evoked a large outwardly rectifying current (**B**) that was completely blocked by capsazepine at all voltages so that only linear leak current remained (**C**), similar to the I/V relationship prior to any additions (**A**). Thus, the complete suppression of the outwardly rectifying I/V relationship by capsazepine strongly suggests the selective expression of TRPV1 channels in this cell line.

Figure 3. Effect of capsazepine on TRPV1 currents evoked by capsaicin.
Upper panel. Long term record from a representative cell of currents sampled at +40mV every 3 seconds (see text for details).
Lower panel -Current traces taken at points A, B and C from the cell held at a potential of -40 mV and ramped from –80 mV to +80 mV over 200 ms with an interpulse interval of 3 seconds.



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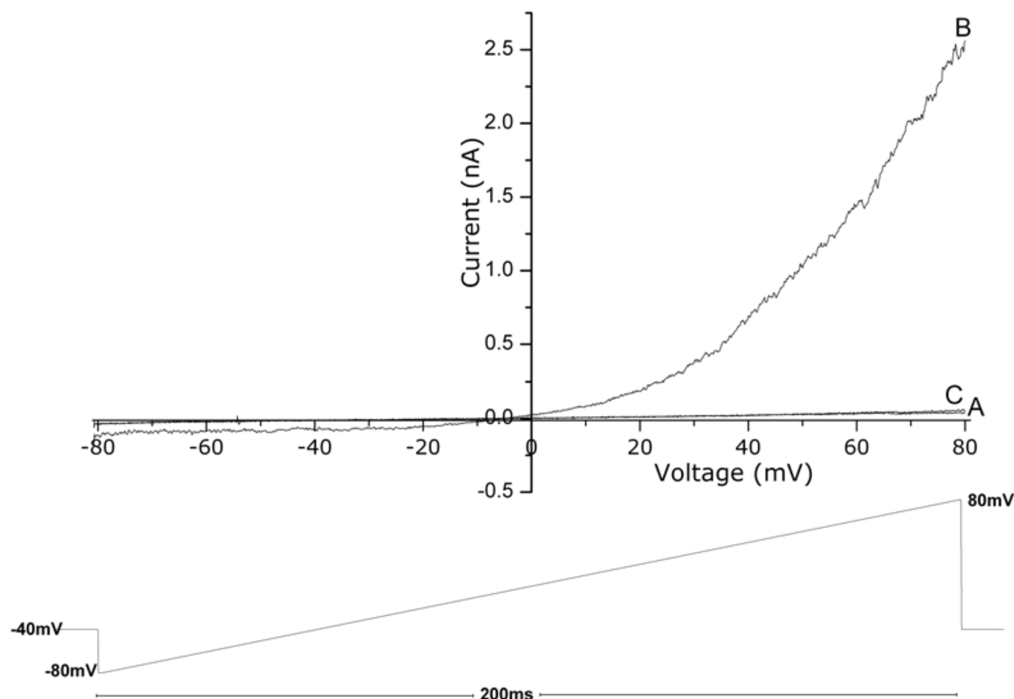
Pharmacology - SB366791:
Even though capsazepine has been used widely in the examination of TRPV1 currents much more potent and selective antagonists have been developed (see review of Szallasi *et al.,* 2007). One particular antagonist, SB-366791, has been shown to block hTRPV1 expressed in HEK293 cells in the low nanomolar range (IC_{50} value around 6 nM, Gunthorpe *et al.,* 2004). This study also reported that the current was blocked to a similar degree over the entire voltage range examined. Thus, in order to provide further evidence for the selective functional expression of TRPV1 channels the effects of 10 nM SB-366791 on capsaicin - evoked responses was investigated using voltage ramps. **Figure 4** shows a typical example where the current evoked by capsaicin (**B**) was completely abolished by SB-366791 (98.66 ± 1.45% inhibition at +80 mV, n=4) so that only residual linear leak current remained (**C**), similar to the I/V relationship prior to any additions (**A**). Evidently, SB-366791 appears more potent than reported previously but given its accepted high selectivity for TRPV1 (Gunthorpe *et al.,* 2004), it provides convincing evidence for selective functional expression in this cell line.

Figure 4. Inhibition of TRPV1 current by SB-366791.
A representative cell held at potential of -40 mV and ramped from −80 mV to +80 mV over 200 ms with an interpulse interval of 3 seconds. The cell was held in control buffer (**A**) and then buffer containing 0.1 µM Capsaicin was perfused over the cell, resulting in increased current (**B**) that was abolished after addition of 10 nM SB-366791 (**C**).



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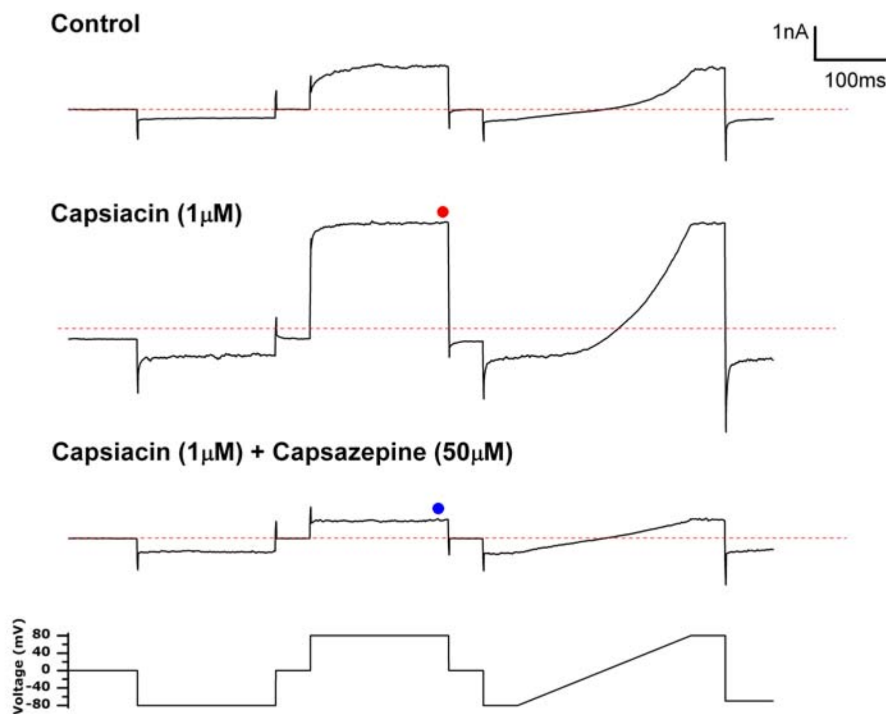
Stability of hTRPV1-HEK293 Cell Line.

IonWorks™ HT Electrophysiology.

In order to monitor the stability of functional expression over time IonWorks™ HT automated electrophysiology was used. Data from a representative cell is shown in **Figure 5**. Currents were evoked using the voltage protocol depicted in the lowest panel of **Figure 5**, before (Control), in the presence of capsaicin (Capsaicin 1 µM) and in the presence of capsazepine that blocked all the capsaicin-evoked current (1 µM Capsaicin + 50 µM Capsazepine). Note that even under control conditions there is some tonic TRPV1 activity, superimposed on the linear leak current, that is subsequently blocked by addition of capsazepine (blue circle). Nonetheless, addition of capsaicin evoked a robust outwardly rectifying current that was quantified by subtracting the current measured at +80 mV in the presence of capsazepine and capsaicin (blue circle) from the current in the presence of capsaicin alone (red circle).

Figure 5. Monitoring TPV1 Expression using IonWorks™ HT.
Currents were evoked using the voltage protocol depicted in the lowest panel before (1st panel, Control), in the presence of capsaicin (2nd panel, Capsaicin) and in the presence of capsiacin and capsazepine (3rd panel). See text for further details).



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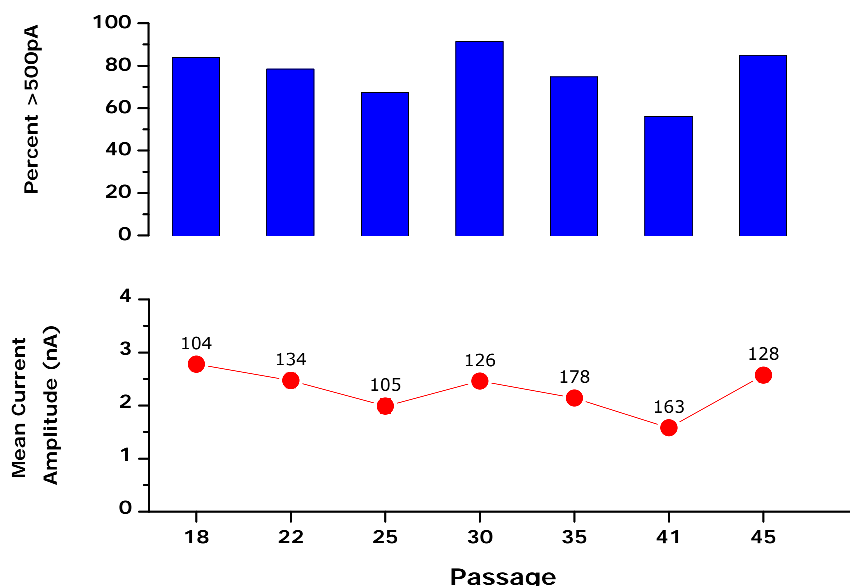
The mean percent of cells expressing capsaicin-evoked currents >500 pA and the mean current amplitude at +80 mV for each cell passage is shown in **Figure 6**. Current amplitudes were typically consistently in excess of 2 nA and expression levels above 60% for > 45 passages.

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 18, 22, 25, 28, 30, 35, 41 and 45. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of cells (numbers above red circles - out of 192 cells for all passages).



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Recommended Culture Conditions:
Cells should be grown in a humidified environment at 37°C under 5% CO_2 using DMEM/F12 (with L-Glutamine) medium supplemented with 10% FBS, 1% Non Essential amino acids, plus 400 µg/ml of Geneticin to ensure that the recombinant expression is maintained.

Transfection of HEK293 cells with the hTRPV1 ion channel does not appear to have altered the growth characteristics of the host cells which exhibited a typical cell division time of 24 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 3-4 days using a seeding density of $1\text{-}1.5\times10^6$ cells per T75 cm^2 or $2\text{-}3\times10^6$ 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), which should be added to the culture vessel or media immediately prior to use.

Media Formulation:

D-MEM/F-12 (with L-Glutamine)	(Invitrogen	#11320)
10% Foetal Bovine Serum	(Invitrogen	#16000)
1% Non Essential amino acids	(Invitrogen	#11140)
400 µg/ml Geneticin (G418)	(Invitrogen	#10131)

Other reagents required:

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

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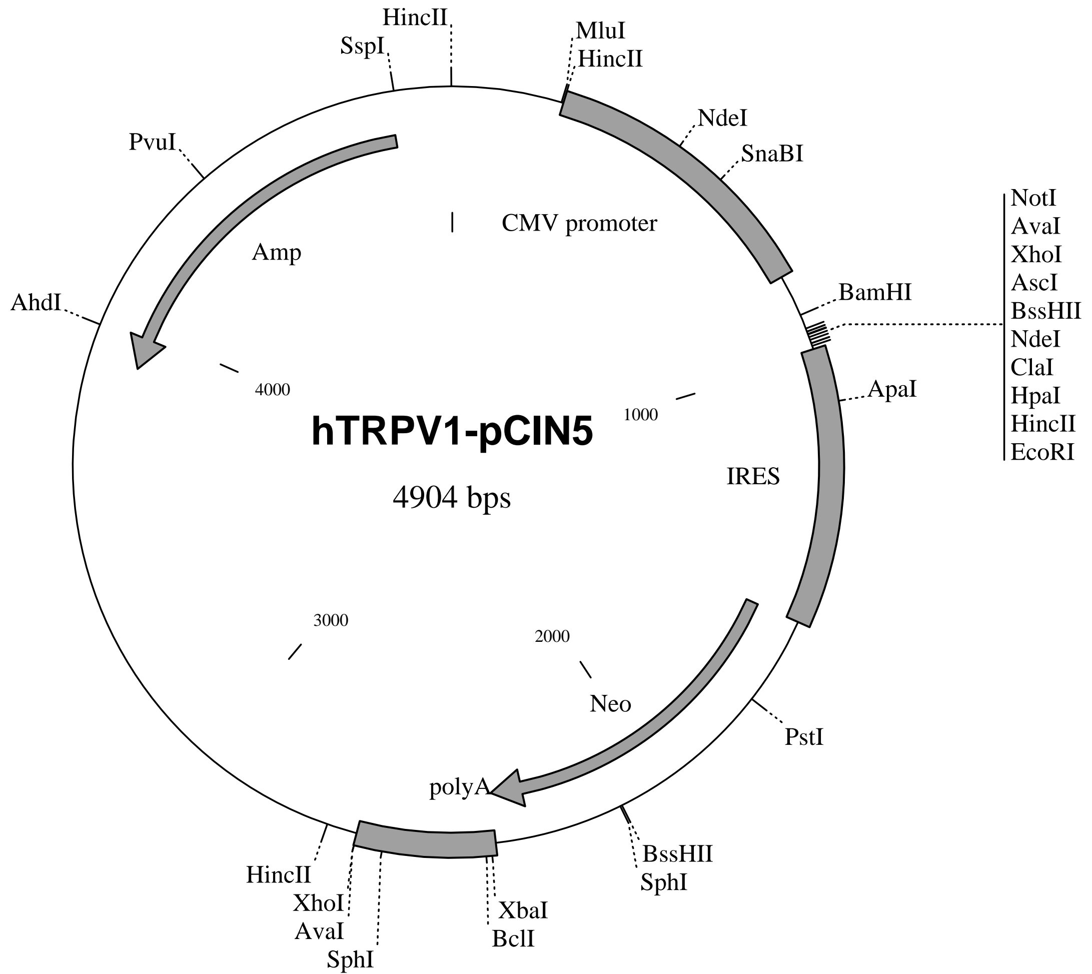
Vector:



Polylinker: CMV-BamHI-NotI-**hTRPV1**-EcoRI-IRES-*neo*

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hTRPV1 Sequence (Accession Number AF196175):

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