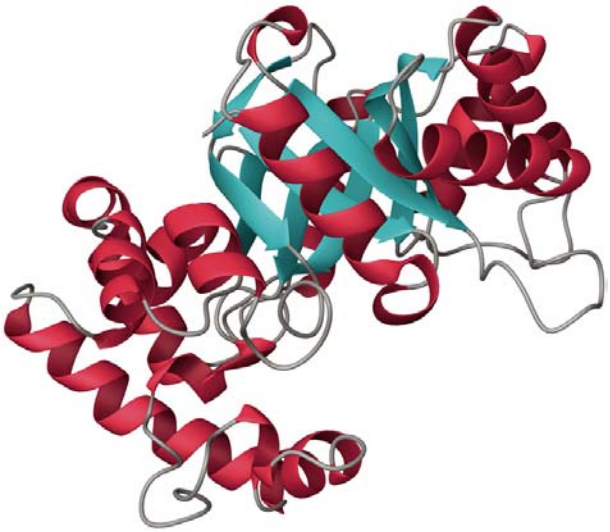
MILLIPORE

hERG-CHO K1 Recombinant Cell Line

cat. #CYL3002

Revision 3



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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.

Product description:

Recombinant CHO-K1 cell line expressing the human ERG (ether-a-go-go related gene) potassium channel.

Format:

2 x 1 ml aliquots containing 1.64 x 10^6 cells/ml in 7.5% DMSO at passage 15 (lot 4).

Mycoplasma Testing:

The cell line has been screened using the ELISA based Mycoplasma Detection kit (Roche) and by a PCR VenorGem kit (Minerva Biolabs) to confirm the absence of Mycoplasma species.

Functional Validation:

CHO-K1 cells stably expressing hERG were analysed 1-2 days after plating by patch-clamp analysis. The cells sealed and patched more easily 2 days post plating giving an average tail current amplitude of between 555±105 pA and 1.3±0.1 nA. 95% of cells expressed the hERG current and the giga-seal obtained was between 80 and 90% with ~70% of cells achieving the whole-cell configuration. IC_{50} values obtained for the known hERG inhibitors were 66 nM for cisapride and 302 nM for terfenadine.

Electrophysiological Properties of the hERG Current

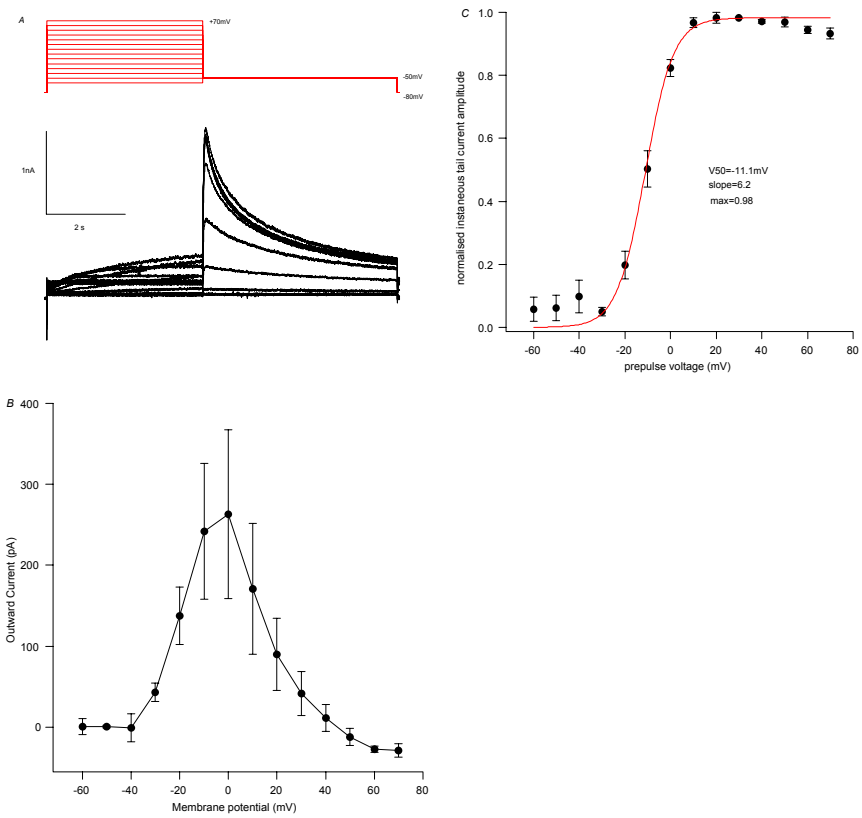

Figure 1. ***A*:** Voltage clamp protocol and currents recorded from hERG-transfected cell. ***B*:** I-V plot of hERG current measures at the end of the depolarising step (mean±sem, *n*=3 cells). ***C*:** Activation curve measured with hERG tail currents and fitted to a Boltzmann function ($V_{1/2}$ = 11.1 mV, *k*=6.2, mean±sem, *n*=3 cells).

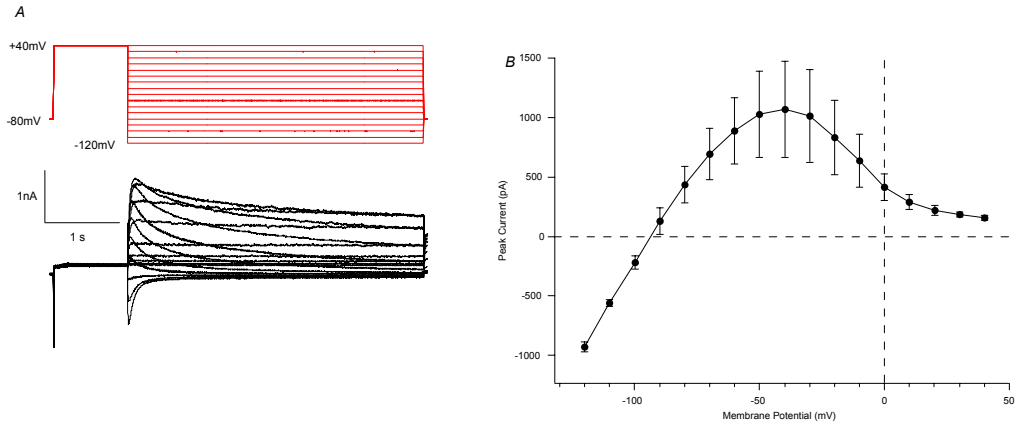

Figure 2. The fully activated I-V relation of the hERG current. ***A*:** Voltage clamp protocol and currents recorded from a hERG-transfected cell. ***B*:** Fully activated I-V plot, maximum current was obtained at voltages between –50 and –40 mV. (mean±sem, *n*=3 cells).

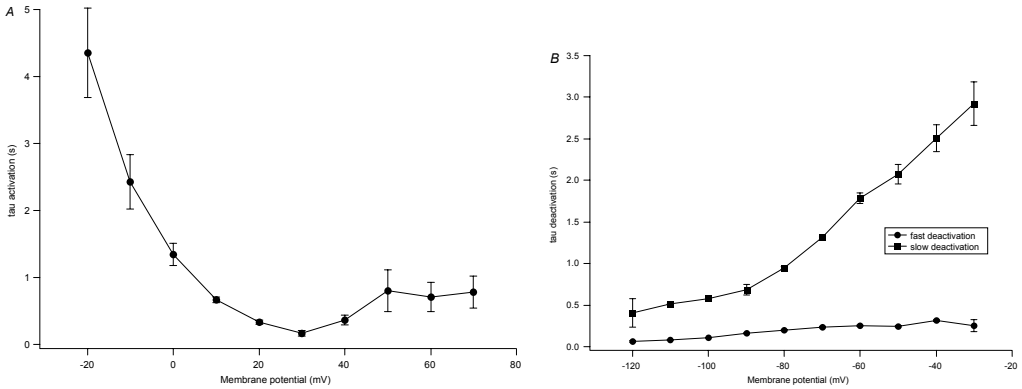

Figure 3. Voltage dependence of activation (*A*) and fast and slow deactivation time constants (*B*). (mean±sem, *n*=3 cells).

Passage number	Mean tail current (nA)	n
13	1.6±0.2	7
14	1.0±0.2	7
17	0.9±0.1	6
19	0.4±0.05	3
25	1.1±0.08	6
26	1.3±0.3	4
28	1.9±0.4	8
29	1.3±0.4	3

Table 1. Summary of the effect of cell passage number on the hERG mean tail current. (mean±sem, n=cells).

Sensitivity to Terfenadine and Cisapride

Concentration of terfenadine	Percentage inhibition of tail current(±sem)	*n*	Concentration of Cisapride	Percentage inhibition of tail current(±sem)	*n*
1nM	8.5±5.1%	3	1nM	14.2±2.5%	4
10nM	16.1±5.7%	3	3nM	9.4±2.4%	4
100nM	34.1±4.3%	3	10nM	33.1±0.6	4
1μM	62.6±7.6%	2	30nM	26±7.0%	5
10μM	91.3±2.5%	3	100nM	50.2±4.0%	3
			300nM	83.4±3.5%	3

Table 2. Summary of the percentage inhibition of hERG tail currents by different concentrations of terfenadine and cisapride. (*n*=number of different cells tested).

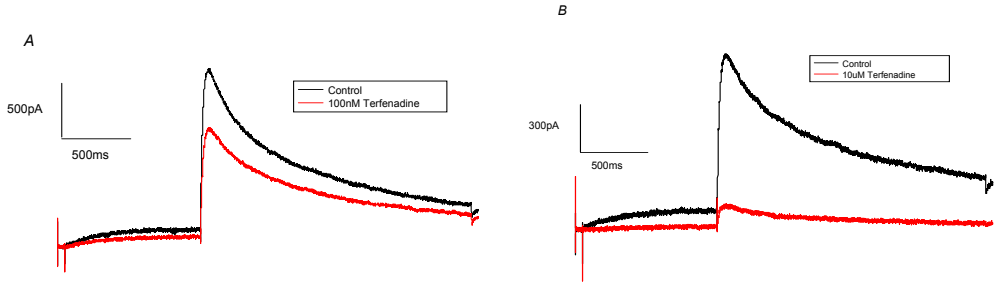

Figure 4. Block of hERG tail currents by terfenadine. Typical recordings showing the effect of 100 nM (*A*) and 10 μM (*B*) terfenadine on tail currents.

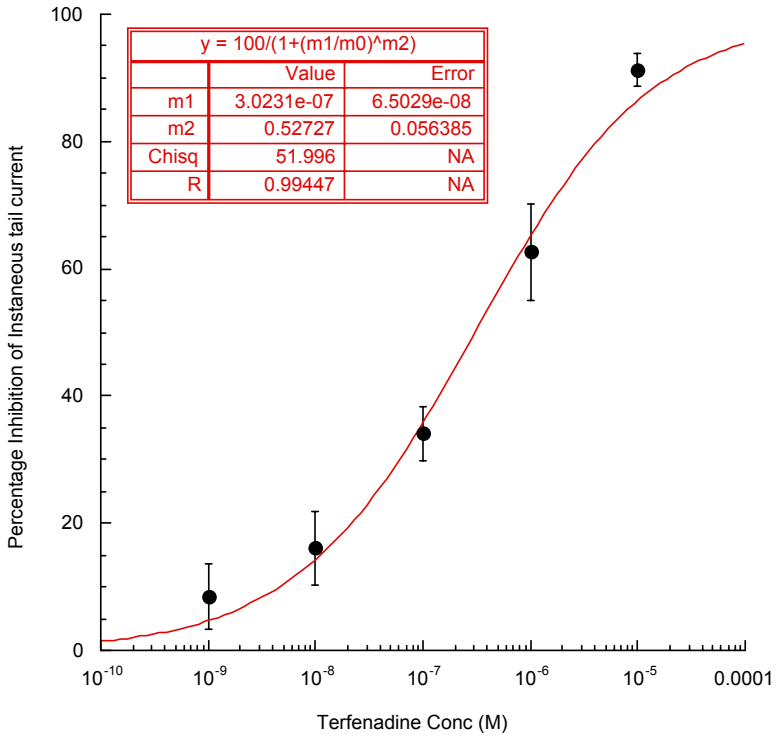

Figure 5. Concentration-response relationship showing the steady-state inhibition of hERG by terfenadine. IC_{50} was calculated by fitting a Logistic function to the data constrained to 100% inhibition. IC_{50} for terfenadine was 302 nM. Data shown is mean±sem (*n*>2 cells).

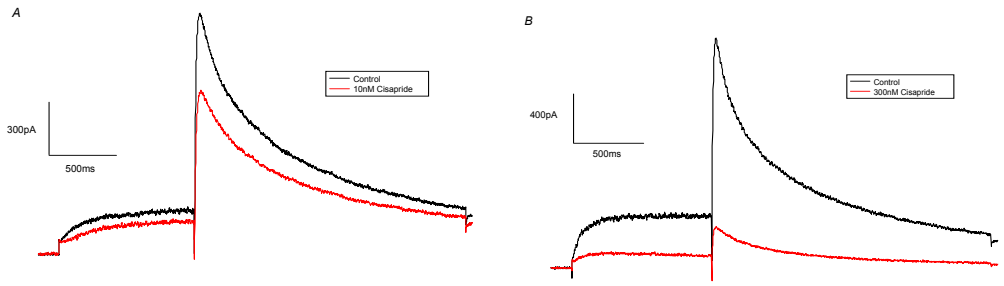

Figure 6. hERG current traces recorded in the presence of 10nM (*A*) and 300 nM (*B*) cisparide. Cells held at –60mV and depolarised to +40 mV for 1 s, and tail currents were recorded with a repolarising step to –50 mV for 2 s.

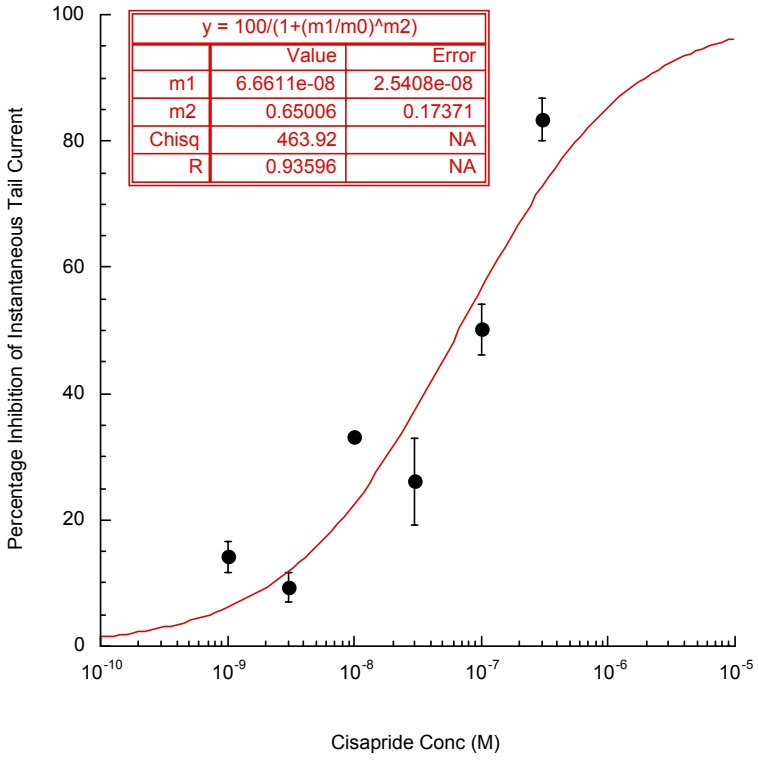

Figure 7. Concentration-response relationship showing the steady-state inhibition of hERG by cisapride. IC_{50} was calculated by fitting a Logistic function to the data constrained to 100% inhibition. IC_{50} for cisapride was 66 nM. Data shown is mean±sem (n>3 cells).

Stability of hERG CHO-K1 Cell Line:

The CHO-K1 cell line expressing hERG, was propagated over 45 days, split every 3 days for 16 passages, in the presence of 200 μg/ml Geneticin and continually evaluated for stability of expression by electrophysiology over this period. There was no significant variation in expression of the hERG current over the 45 days of continuous culture under the recommended growth conditions.

Recommended culture Conditions:

Cells should be grown in a humidified environment at 37°C under 5% CO_2 using HamsF12+Glutamax medium supplemented with 10% FBS plus 200 μg/ml of Geneticin to ensure that the recombinant expression is maintained

Transfection of CHO-K1 cells with the hERG 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 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 0.5-1×10^6 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 (200 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)
200 μg/ml Geneticin (G418)	(Invitrogen	#10131)

Other reagents required:

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

Vector:

pWizTag (a modified version of gWiz 3.0, Gene Therapy Systems)

hERG Sequence (Accession Number U04270):

ATGCCGGTGCGGAGGGGCCACGTCGCGCCGCAGAACACCTTCCTGGACACCATCATCCGCAAGTTTGAG
GGCCAGAGCCGTAAGTTCATCATCGCCAACGCTCGGGTGGAGAACTGCGCCGTCATCTACTGCAACGAC
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