



DATASHEET

PrecisION™

hERG-HEK

Recombinant Cell Line

CATALOG # CYL3006

REVISION # M07

**ORDERING INFORMATION
AND TECHNICAL SERVICES**

Millipore (UK) Ltd
6-7 Technopark
Cambridge CB5 8PB
UK

Tel: +44 (0) 1223 508191

Fax: +44 (0) 1223 508198

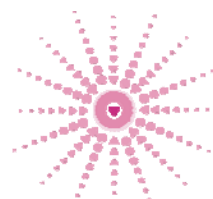
CUSTOMER SERVICES

UK: 0800 0190 333

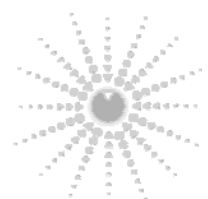
US: 800 437 7500



GPCR



Ion Channels



**Kinase /
Phosphatase**



**Safety & Toxicity
Profiling**

Product description:

Recombinant HEK293 cell line expressing the human ERG (ether-a-go-go related gene) potassium channel (accession number U04270).

Format:

2 x 1 ml aliquots containing 1.00×10^6 cells/ml in 10% DMSO at passage 11 (Lot 2).

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:

HEK293 cells stably expressing hERG were analysed 2-3 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 1.04 ± 0.19 nA and 1.90 ± 0.38 nA. 96% of cells expressed the hERG current and the giga-seal was obtained in over 90% of cells with over 90% of cells achieving the whole-cell configuration. IC_{50} values obtained for the known hERG inhibitors were 12 nM for cisapride and 5.3 nM for terfenadine.

Recommended Culture Conditions:

Recommended culture conditions and standard operating procedure are provided with the product.

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.

Electrophysiological Properties of the hERG Current.

Figure 1: **A:** Voltage-clamp protocol currents recorded from a hERG-transfected cell. **B:** I-V plot of hERG current measured at the end of the depolarising step (mean ± SEM; n=6 cells). **C:** Activation curve measured with hERG tail currents and fitted to a Boltzmann function ($V_{1/2} = -11.9 \pm 3.6$ mV and $k = 6.7 \pm 0.1$, mean ± SEM, n = 6 cells).

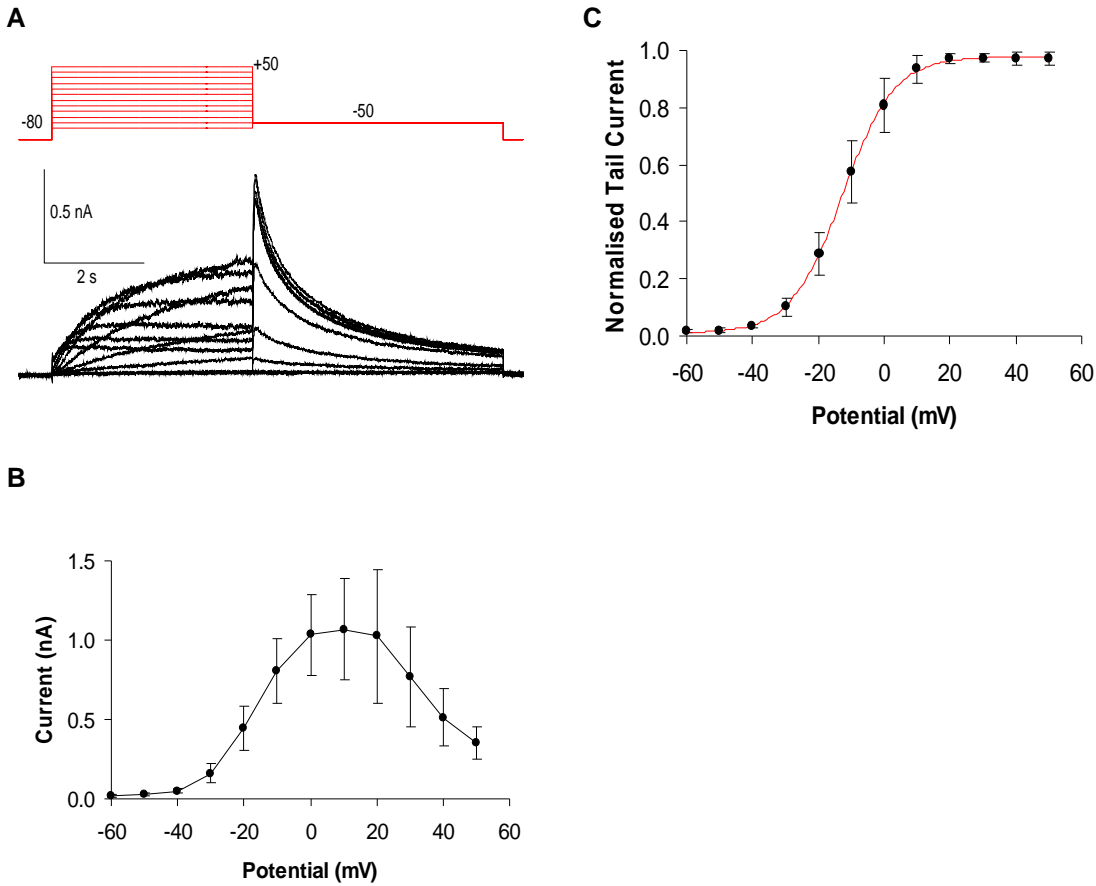


Figure 2: The fully activated I-V relation of the hERG current. **A:** Voltage clamp protocol and currents recorded from a hERG-transfected cell. **B:** Fully activated I-V plot, maximum current was obtained at voltages between -40 and -20 mV, (mean ± SEM; n = 4 cells).

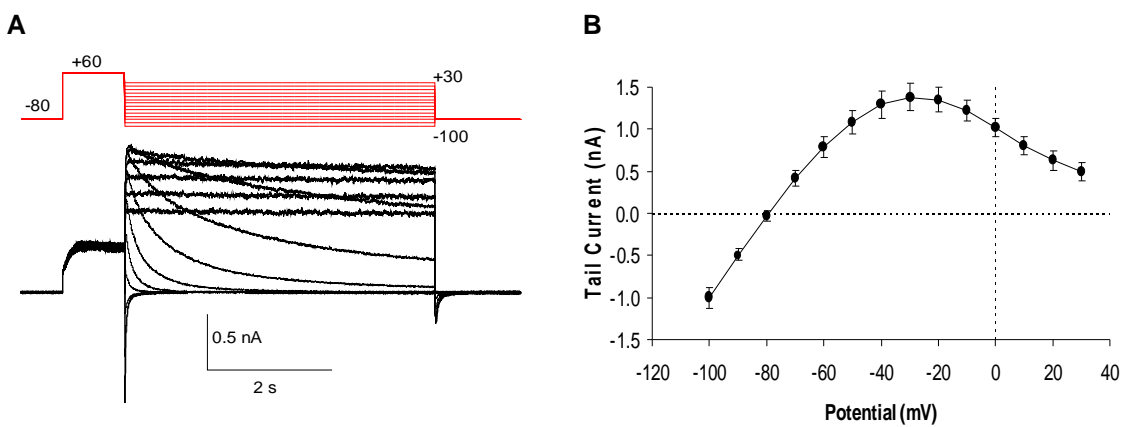
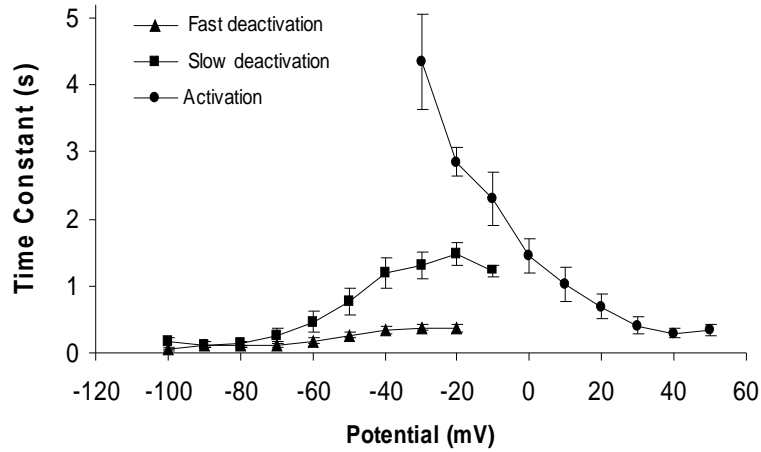


Figure 3: Voltage dependence of hERG current activation and deactivation. The activation time course was measured by fitting the rising phase of current traces from Figure 1 with a mono exponential function. The hERG deactivation fast and slow time constants were obtained by fitting the decay of tail currents from Figure 2 with a double exponential function.



Pharmacology - E4031, Verapamil, Terfenadine and Cisapride:

Summary of the percentage inhibition of hERG tail currents by different concentrations of terfenadine and of cisapride.

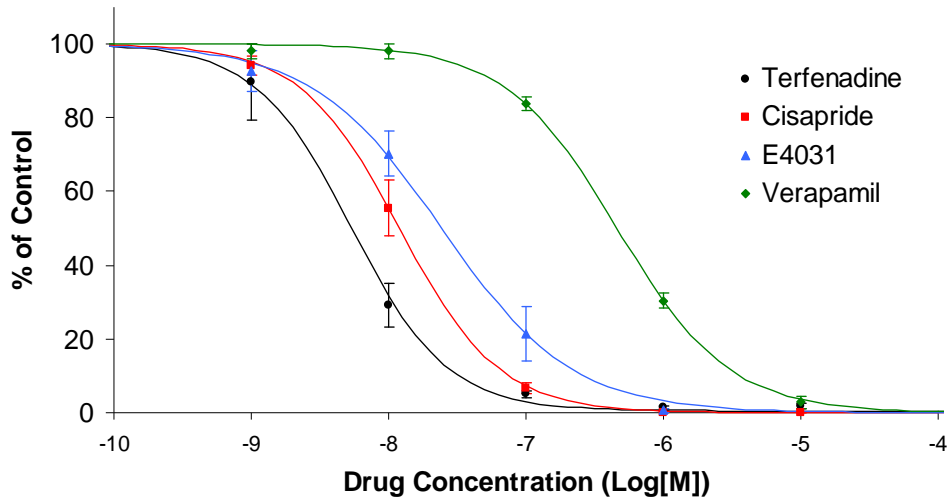
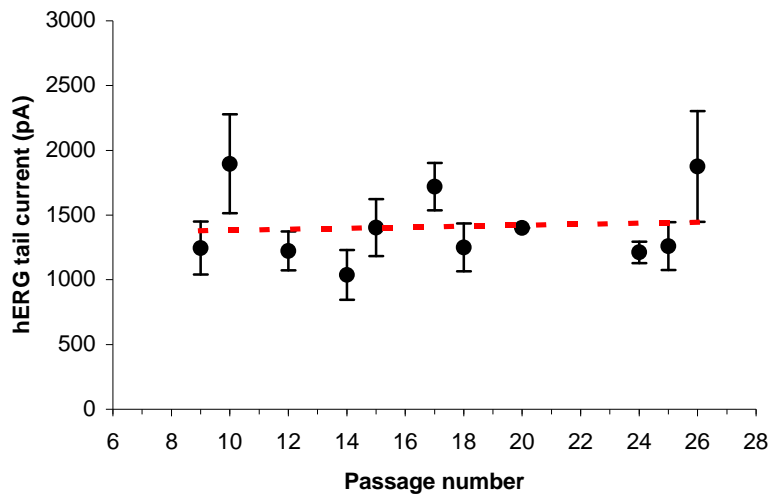


Figure 4: Concentration-response relationship showing the steady-state inhibition of hERG by the inhibitors terfenadine, cisapride, E4031 and verapamil. IC_{50} values were calculated by fitting a Logistic function to the data, constrained to 100% relative current. The IC_{50} values were: Terfenadine, 5 nM; Cisapride, 12 nM; E4031, 23 nM; Verapamil, 360 nM. Data shown is mean \pm SEM, (n = 3-6 cells).

Stability of hERG Cell Line:

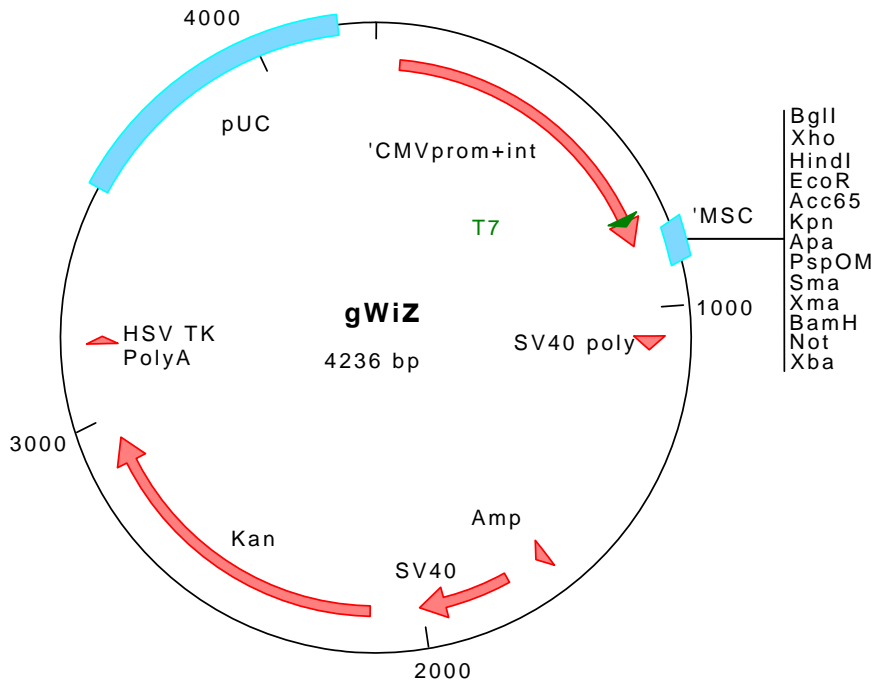
Figure 5. The hERG-HEK293 cell line was propagated over >124 days, split 1-2 times a week for 26 passages in the presence of 400 µg/ml Geneticin and continually evaluated for stability of expression by electrophysiology over this period. There was no significant variation in expression of the hERG current over the 124 days of continuous culture under the recommended growth conditions.

A change in growth pattern may be observed at around passage 32 when the cells may begin to grow in clumps. This change in the cells' growth pattern is accompanied by a concomitant decrease in the hERG current. As a result we would not recommend using these cells past passage 30 for electrophysiological purposes.



Passage Number	Mean Tail Current (pA)	SEM	n=(number of cells)
9	1246	205	6
10	1896	381	10
12	1223	151	10
14	1038	193	4
15	1404	219	8
17	1720	183	16
18	1250	185	4
20	1400	0	1
24	1213	83	4
25	1260	186	5
26	1875	427	8

Vector:
gWiz 3.0 (Gene Therapy Systems)



MCS sequence of the gWiz3.0 vector (801-960)

GTCTTTTCTGCAGGCACCGTCGACTTAAC AGATCTCGAGCTC AAGCTTC GAATTC
 Kpn I Apa I Sma I BamH I
 Not I
 TGCAGTCGAC GGTACCGCGGG CCCGGGATCCACCGGGTACAAGTAA GCGGCCGC
 Xba I*
 GAC ICTAGATCATAATCAGCCATACCATTTGTAGAGGTTTTACTTGCT

* Blocked by overlapping *dam* methylation

hERG Sequence (Accession Number U04270):**References:**

Crumb, W.J. Jr (2000) Loratadine blockade of K⁺ channels in human heart: comparison with terfenadine under physiological conditions. *J Pharmacol. Exp. Ther.* **292(1)**: 261-4.

Mohammad, S., Zhou, Z., Gong, Q. and January, C.T. (1997) Blockage of the HERG human cardiac K⁺ channel by the gastrointestinal prokinetic agent cisapride. *Am J Physiol.* **273(5 Pt 2)**: H2534-8.

Rampe, D., Roy, M.L., Dennis, A., and Brown, A.M. (1997) A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett.* **417(1)**: 28-32.

Sanguinetti, M.C., Jiang, C., Curran, M.E. and Keating, M.T. (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. *Cell* **81(2)**: 299-307.

Snyders, D.J. and Chaudhary, A. (1996) High affinity open channel block by dofetilide of HERG expressed in a human cell line. *Mol Pharmacol.* **49(6)**: 949-55.

Zhou, Z., Gong, Q., Ye, B., Fan, Z., Makielski, J.C., Robertson, G.A. and January, C.T. (1998) Properties of HERG channels stably expressed in HEK293 cells studied at physiological temperature. *Biophys J.* **74(1)**: 230-41.