



DATASHEET

PrecisIOn™

hHCN4-CHO

Recombinant Cell Line

CATALOG # CYL3012

REVISION # M08

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

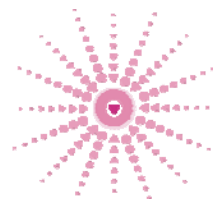
CUSTOMER SERVICES

UK: 0800 0190 333

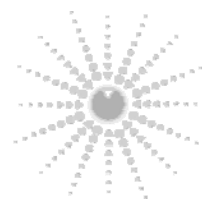
US: 800 437 7500



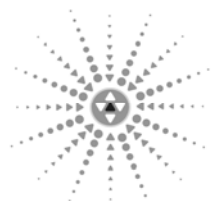
GPCR



Ion Channels



Kinase /
Phosphatase



Safety & Toxicity
Profiling

Product description:

Recombinant CHO-K1 cell line expressing the human HCN4 (hyperpolarization activated cyclic nucleotide-gated potassium channel 4).

Format:

2 x 1 ml aliquots containing 1.25×10^6 cells/ml in 10% DMSO at passage 14.

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 hHCN4 were analysed by IonWorks HT. Over 88% of cells expressed >300 pA outward current (n=1099) with a mean current amplitude of $1360 \text{ pA} \pm 0.03$ (n=971). The HCN4 current was inhibited by standard blockers such as capsazepine ($\text{pIC}_{50} = 4.2 \pm 0.1$; n=3) and ZD-7288 ($\text{pIC}_{50} = 4.4 \pm 0.2$; n=4).

Recommended Culture Conditions:

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

Licensing Statements

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.

The bovine growth hormone (bgh) polyadenylation signal and its use in the expression of recombinant proteins is covered by claims listed in U.S. Patent No. 5,122,458, EU Patent No. 0 173 552 and Japanese Patent No. 1955752 (collectively "CLAIMED DNA and/or CLAIMED CELLS") owned and licensed to Millipore (formerly Upstate Biotechnology Inc.) by Research Corporation Technologies, Inc., 101 North Wilmot Road, Suite 600, Tucson, AZ 85711-3335 ("RCT").

Use of this technology is restricted to research purposes only. The purchased/licensed cell line and all bacteria, phages and plasmids derived from this cell line, in whole or in part, and all proteins expressed from the cell line shall be used for research uses only. "Research purposes" means uses directed to the identification of useful recombinant proteins and the investigation of the recombinant expression of proteins. In no event shall research use include any of the following: any use in humans of a CLAIMED DNA or CLAIMED CELL; any use in human or protein expression or other substance expressed or made at any stage of its production that use the CLAIMED DNA or a CLAIMED CELL; or any use in which a CLAIMED DNA or CLAIMED CELL would be sold or transferred to a third party. No license, other than research use, is expressed or implied by the purchase/license of the cell line. By accepting or using Millipore's cell line product, you agree to be bound for the following use/license restrictions:

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IonWorks™ HT is a trademark of Molecular Devices Corporation

Electrophysiological Properties of the hHCN4 current.**Figure 1.** IonWorks HT recording from hHCN4-CHO K1 cells.

A. Representative currents evoked from the voltage-command shown in the upper panel. Note the slowly activating inward current in response to hyperpolarising steps. Calibration bars 2 s and 500 pA.

B. Population histogram for peak (inward) current amplitude at -110 mV obtained from 1099 cells.

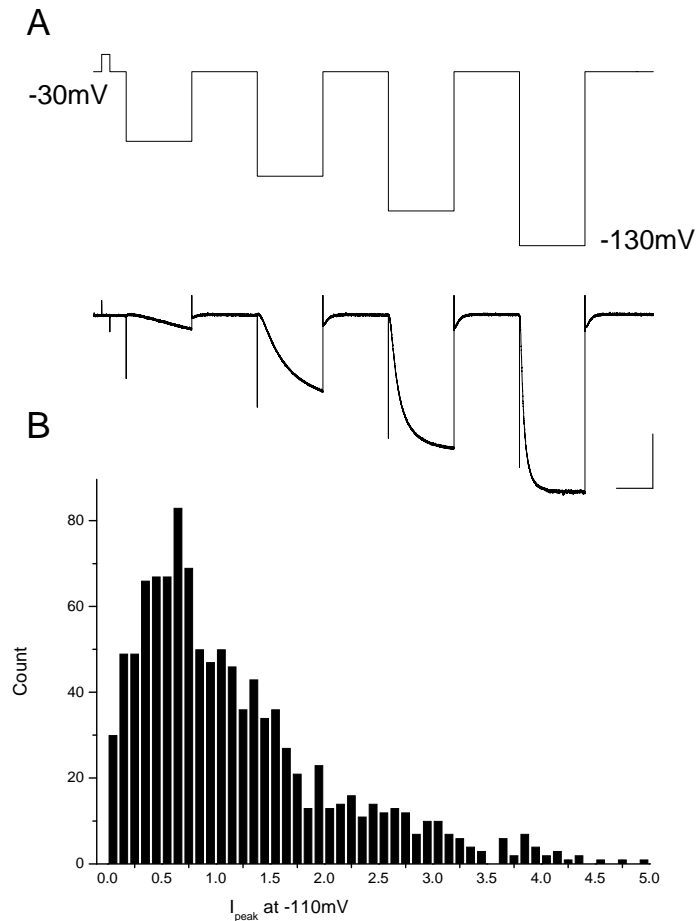


Figure 2. Stability of expression over passage (mean peak current for expressors (blue triangles) and % of cells expressing (red circles)). Each point represents the results from a single IonWorks HT run.

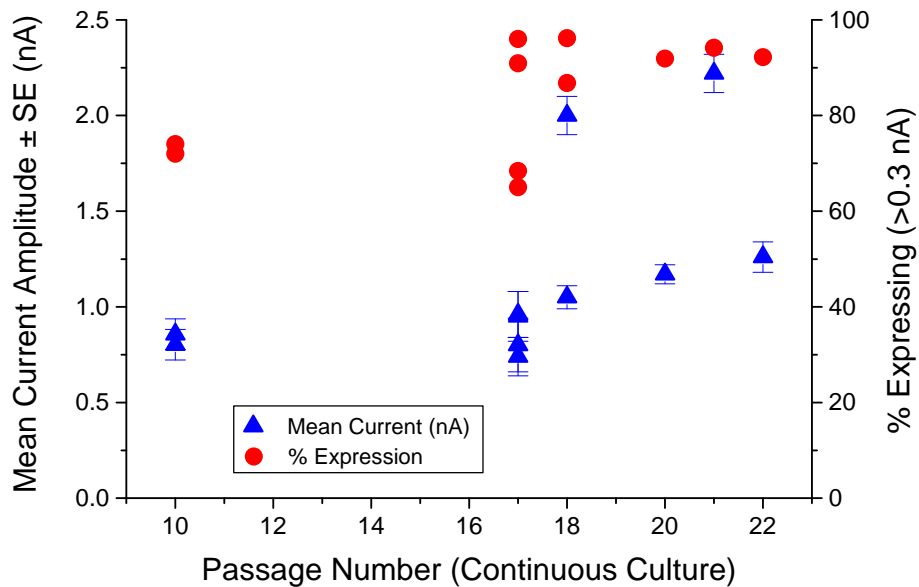
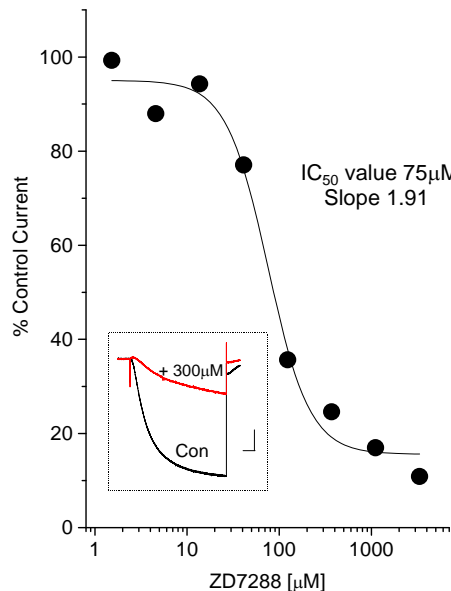


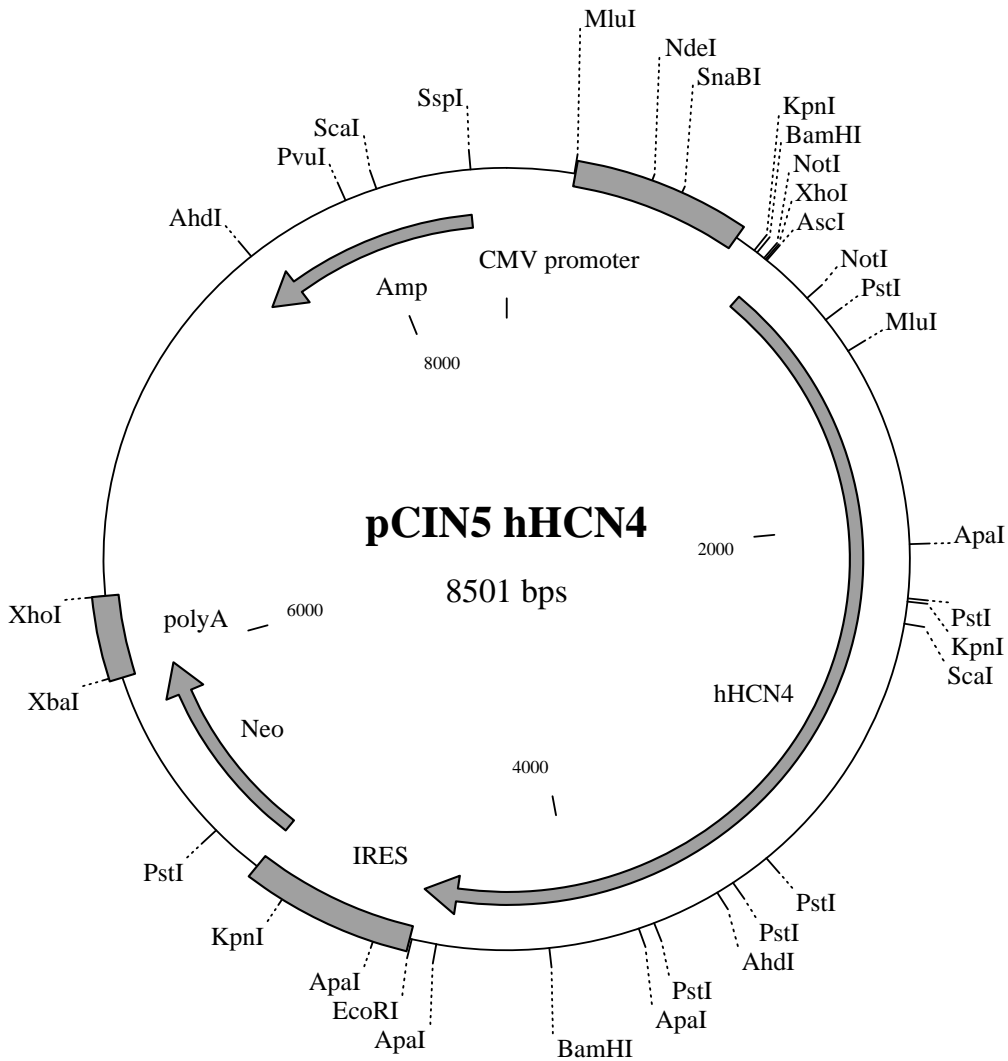
Figure 3. Concentration-response curve and representative current record (inset) for inhibition of hHCN4 currents by ZD7288 in stably expressing CHO-K1 cells. The abscissa shows the log concentration of ZD7288 (μM) and the ordinate the current amplitude normalised to the pre-drug value. Each point represents the median value from 2-4 determinations (different cells). From the four parameter logistic equation the IC_{50} value was $75 \mu\text{M}$ and the Hill slope 1.91. hHCN4 currents were evoked by a test step of 4 s from -30 mV to -110 mV . The scale bars in the inset are 500 ms (x) and 500 pA (y), respectively.



Stability of hHCN4-CHO K1 Cell Line:

The hHCN4-CHO K1 cell line has stable expression for >25 passages.

Vector:



Polylinker: CMV-BamHI-NotI-AscI-hHCN4-EcoRI-IRES-neo

hHCN4 Sequence:

The sequence of hHCN4 used to create this stable cell line encodes a protein with the following change to that encoded by the GenBank accession number AJ238850: Thr110Ser.