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1.0 OVERVIEW OF HERG T-REX™ CHO CELLS

The hERG gene encodes a potassium channel expressed in the mammalian heart; activity of this ion channel is crucial for repolarization and relaxation of cardiac muscle during every heartbeat. Potassium efflux occurs when the channel is open and the cardiac myocyte membrane potential is positive to the equilibrium potential for potassium—roughly -90 mV. Human mutations of this gene increase susceptibility to QT-interval prolongation as determined on an EKG; this prolonged interval can lead to ventricular arrhythmias that are lethal if untreated. Carriers may be asymptomatic until a sudden startle stimulus (e.g., an alarm or telephone call at an unexpected time) causes fainting (if awake) or sudden onset of ventricular arrhythmia.

Additionally a wide variety of drugs from diverse chemical scaffolds block this channel. Patients admitted for QT-interval prolongation or ventricular arrhythmia are typically screened for medications. In several cases subsequent patch-clamp experiments demonstrate that a therapeutically relevant level of a prescription drug in a patient blocks hERG channels expressed in recombinant cell lines. Such findings have lead to the withdrawal of 10-20 marketed drugs, and a recommendation from the ICH that all new drugs be tested in such patch-clamp assays to assess hERG block liability before they are administered to humans.

2.0 MATERIALS SUPPLIED

Cell Line Name:	hERG T-REx™ CHO
Description:	hERG T-REx™ CHO cells were generated by transfection of the hERG coding sequence in the Tet-regulated expression vector **pT-REx-DEST30** into cells expressing the Tet-repressor (T-REx™ CHO, Invitrogen Cat. No. R718-07) to generate hERG T-REx™ CHO clone 40 inducibly expressing robust levels of hERG activity. Cells are resistant to blasticidin , and are maintained using DMEM media with Geneticin added to maintain transgene activity during culture. Tetracycline (or doxycycline) is added to 1 micrograms per ml for induction of hERG expression.
Product Number:	K1237
Shipping Condition:	Dry Ice
Storage Condition:	Liquid nitrogen. **Immediately upon receipt, cells must be stored in liquid nitrogen or thawed for immediate use. Cells stored at -80°C can quickly lose viability.**
Quantity:	~2,000,000 ($2x10^6$ cells/mL)
Application:	Detection of antagonists of the hERG ion channel.
Growth Properties:	Adherent
Cell Phenotype:	Epithelial
Selection Marker:	Blasticidin (10 µg/mL) Geneticin 400 µg/mL
Vector Used:	**pT-REx-DEST30**
***Mycoplasma* Testing:**	Negative
Biosafety Level:	1

3.0 MATERIALS REQUIRED, BUT NOT SUPPLIED

Media/Reagents	Recommended Source	Part #
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648-010
DMEM with GlutaMAX™	Invitrogen	10569-010
Fetal bovine serum (FBS), dialyzed (**DO NOT SUBSTITUTE**)	Invitrogen	26400-036
Non-essential amino acids (NEAA)	Invitrogen	11140-050
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140-122
Dulbecco's Phosphate Buffered Saline (PBS)	Invitrogen	14190-144
HEPES (1 M, pH 7.3)	Invitrogen	15630-080
0.05% Trypsin/EDTA	Invitrogen	25300-062
Blasticidin (antibiotic)	Invitrogen	R210-01
Geneticin (antibiotic)	Invitrogen	10131-035
Trypan Blue	Invitrogen	15250-061
Doxycycline hydrochloride	MP Biomedicals	195044

Consumables	Recommended Source	Part #
47 mm^2 Petri Plate	Various	—
Cover Slips, #1 5 mm round	Warner Instruments	64-0700
Borosicilate glass pipettes	Various	

Equipment	Recommended Source
Inverted microscope equipped with phase-contrast capabilities	Various
Pipette puller	Various
Microforge	Various
Patch-clamp amplifier	Various
Micromanipulator	Various

3.1 Optional Equipment and Materials

- None

4.0 CELL CULTURE CONDITIONS

4.1 Media Required

Component	Growth Medium	Freezing Medium
DMEM with GlutaMAX™	90%	—
Dialyzed FBS	10%	—
NEAA	0.1 mM	—
HEPES (pH 7.3)	25 mM	—
Penicillin (antibiotic)	100 U/mL	—
Streptomycin (antibiotic)	100 µg/mL	—
Blasticidin (antibiotic)	10 µg/mL	—
Geneticin (antibiotic)	400 µg/mL	--
Recovery™ Cell Culture Freezing Medium	—	100%

Note: Unless otherwise stated, have all media and solutions at least at room temperature (we recommend 37°C for optimal performance) before adding them to the cells.

4.2 Growth Conditions

For detailed cell growth and maintenance directions, see **Section 7.0**.

1. Thaw cells in Growth Medium without Blasticidin and Geneticin and culture them in Growth Medium with Blasticidin and Geneticin after the first passage. Pass cells twice per week (Monday and Thursday) at 1:6 and 1:8, respectively. Maintenance of confluence below between 10 and 80% is suggested for optimal hERG expression. Do not allow cells to reach confluence, or hERG expression level may decrease.
2. Freeze cells at 2 x 10^6 cells/mL in Freezing Medium.

5.0 ASSAY PROCEDURE

The following instructions outline the general procedure for using hERG T-REx™ CHO cells in patch-clamp assays to measure current block. Cells are plated onto suitable coverslips at 200-400K cells per 47 mm^2 dish 48 hr before the assay, and induced with doxycycline at 1 µg/mL. The current can be measured from a 12-point current-voltage (I-V) protocol described below; appropriate block protocols can be designed and utilized by the user.

5.1 Detailed Assay Protocol

Note: Some solvents may affect assay performance. Assess the effects of solvent before screening.

5.2.1 Precautions

- It is assumed the user will employ good patch-clamp assay methods to obtain high-quality, high-resistance seals and to obtain whole-cell access and stable recording conditions.

5.2.2 Harvest and plate cells

1. Remove media & rinse cells with 10 mL PBS.
2. Lift cells with 2-5 mL 0.05% Trypsin/EDTA.
3. Neutralize trypsin/EDTA by addition of an additional 3 mL medium.
4. Triturate cells 10-15 times and then count on hemacytometer.
5. Dilute with medium to desired density (200-400K cells per 3 mL).
6. Add diluted cells to 47mm dish containing 8-10 5mm round coverslips.
7. Return 47 mm dish to #&C incubator; allow cells to adhere to coverslips overnight.

5.2.3 Prepare Stock Solutions

Prepare compound test solutions as required.

5.2.4 Patch-clamp cells and determine current-voltage curve

1. Remove coverslip from 47 mm^2 plate in incubator and place on microscope stage in bath chamber; perfuse with PBS or equivalent at 1 ml/min.
2. Visually select a cell and under visual control manipulate pipette to cell plasma membrane. Monitor seal resistance; obtain G Ω seal.
3. Obtain whole-cell recording mode.
4. Set holding potential to -90 mV; sample current at 2.0 kHz and filter at 667 Hz or equivalent.
5. Voltage-dependent activation curves are measured by stepping the command potential to -70 mV for 50 ms, then stepping the command potential through the range of -70 to +40 mV in 10 mV increments for a duration of 2 s, returning to the command potential to -70 mV for 2 s and then returning to the holding potential of -90 mV, every 5 s.
6. The hERG current elicited by the series of depolarizing pulses is measured during the -70 mV repolarization phase from the peak outward going current, or tail current. This peak tail current should reach a maximum following the voltage-step to +40 mV

6.0 REPRESENTATIVE DATA

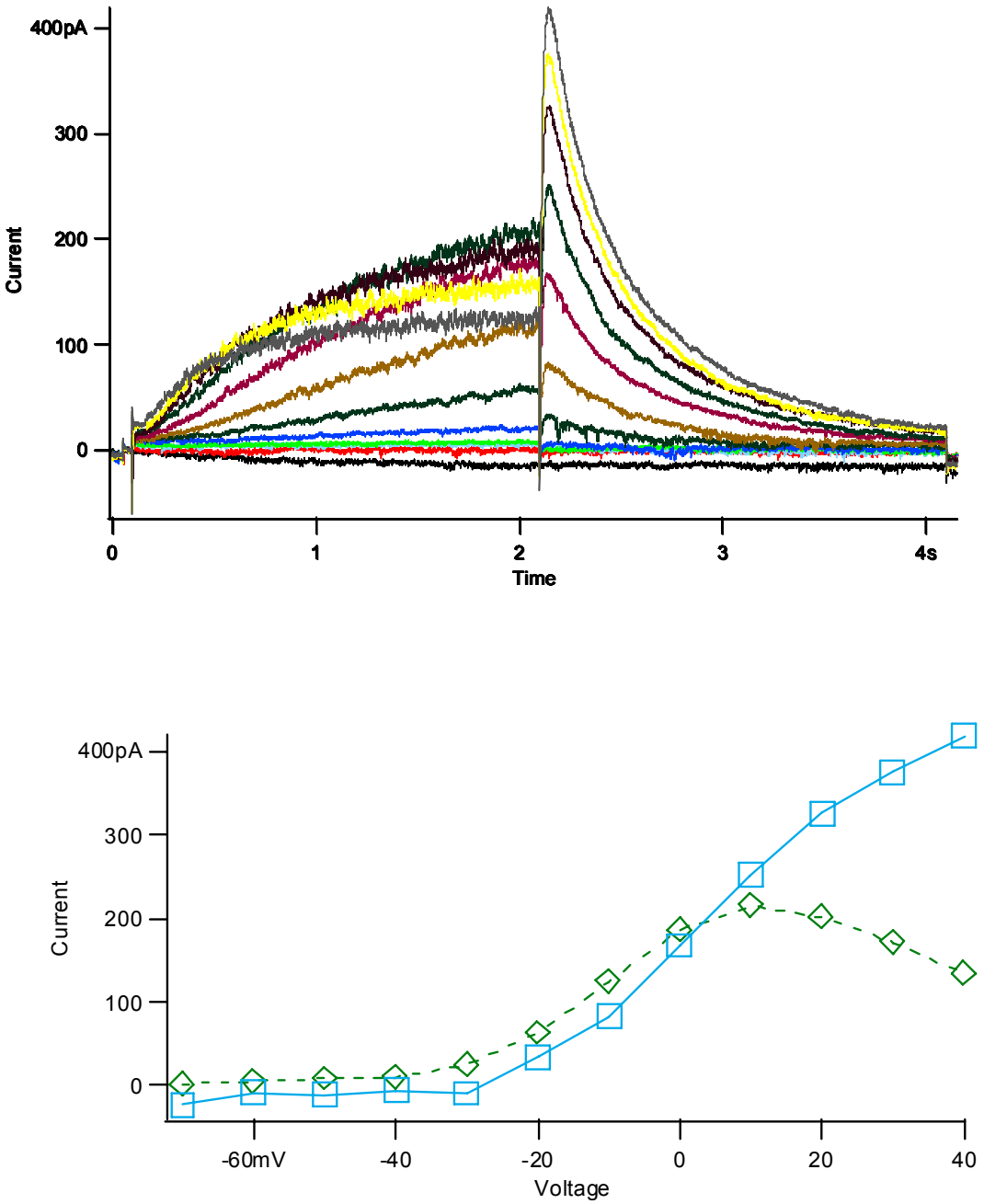

Figure 1. Example of currents recorded from hERG T-REx™ CHO ® cells in patch-clamp assay. Top: Cells were held at -90 mV and stepped in 10 mV increments from -7- to +40 mV, then back to -70 mV to elicit tail currents. Bottom: the peak current during the step (diamonds) and during the step back to -70 mV (the tail current; boxes) are plotted.

7.0 DETAILED CELL HANDLING PROCEDURES

7.1 Thawing Method

1. Place 14 mL of Growth Medium without Blasticidin or Geneticin into a T75 flask.
2. Place the flask in a humidified 37°C/5% CO_2 incubator for 15 minutes to allow medium to equilibrate to the proper pH and temperature.
3. Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.
4. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
5. Transfer the vial contents drop-wise into 10 mL of Growth Medium without Blasticidin or Geneticin in a sterile 15-mL conical tube.
6. Centrifuge cells at 200 × *g* for 5 minutes.
7. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh Growth Medium without Blasticidin or Geneticin.
8. Transfer contents to the T75 tissue culture flask containing pre-equilibrated Growth Medium without Blasticidin or Geneticin and place flask in a humidified 37°C/5% CO_2 incubator.
9. At first passage, switch to Growth Medium with Blasticidin and Geneticin.

7.2 Propagation Method

1. Cells should be passaged or fed at least twice a week. Cells should be maintained between 10% and 80% confluence. Do not allow cells to reach confluence.
2. To passage cells, aspirate medium, rinse once in PBS, add Trypsin/EDTA (3 mL for a T75 flask and 5 mL for a T175 flask and 8 mL for T225 flask) and swirl to coat the cells evenly. Cells usually detach after ~2–5 minutes exposure to Trypsin/EDTA. Add an equal volume of Growth Medium to inactivate Trypsin.
3. Verify under a microscope that cells have detached and clumps have completely dispersed.
4. Spin down cells and resuspend in Growth Medium.

7.3 Freezing Method

1. Harvest the cells as described in **Section 7.2**. After detachment, count the cells, then spin cells down and resuspend in 4°C Cell Culture Freezing Medium to $2x10^6$ cells/mL.
2. Dispense 1.0-mL aliquots into cryogenic vials.
3. Place in an insulated container for slow cooling and store overnight at –80°C.
4. Transfer to liquid nitrogen the next day for storage.

8.0 REFERENCES

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Zhou, Z. *et al*., **Biophysical Properties of HERG Channels Stably Expressed in HEK293 Cells Studied at Physiological Temperature**, (1998) *Biophys J*. **74**:p230-241.

9.0 PURCHASER NOTIFICATION

Limited Use Label License No. 51: Blasticidin and the Blasticidin Selection Marker

Blasticidin and the blasticidin resistance gene (bsd) are the subject of U.S. Patent No. 5,527,701 sold under patent license for research purposes only. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

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Use of Genetically Modified Organisms (GMO)

Information for European Customers hERG T-REx™ CHO cells cell line is genetically modified with the plasmid **pT-REx-DEST30-hERG**. As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.