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1.0 DESCRIPTION

hERG-T-REx™ 293 DA (Division-arrested) cells and hERG-T-REx™ 293 cells contain the human ether a go go related gene KCNH2) stably integrated into the cell line. hERG-T-REx™ 293 DA cells and hERG-T-REx™ 293 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™ 293, Invitrogen cat. no. R710-07) to produce cells that can be induced to express large hERG currents. Dividing cells are maintained in culture using DMEM medium with Blasticidin and Geneticin® added to maintain transgene activity and expression of the Tet-repressor and the hERG channel. Dividing cells require the addition of doxycycline (or tetracycline) to 1 µg per ml for induction of hERG expression; division-arrested cells were induced prior to arrest.

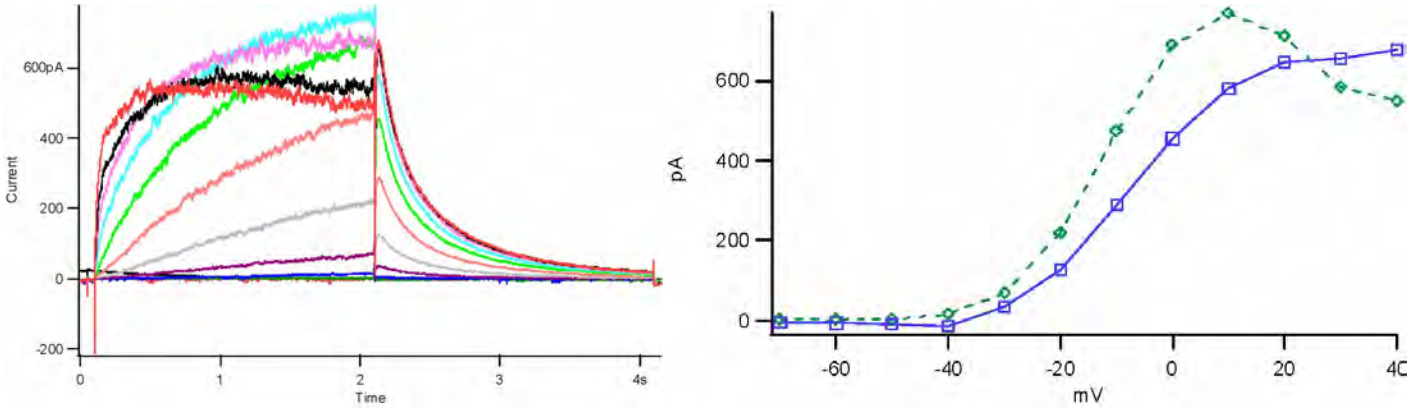

Figure 1. Example of currents recorded from dividing hERG-T-REx™ 293 cells in patch-clamp assay. Left: Cells were held at -90 mV and stepped in 10 mV increments from -7- to +40 mV, then back to -70 mV to elicit tail currents. Right: the peak current during the step (diamonds) and during the step back to -70 mV (the tail current; boxes) are plotted.

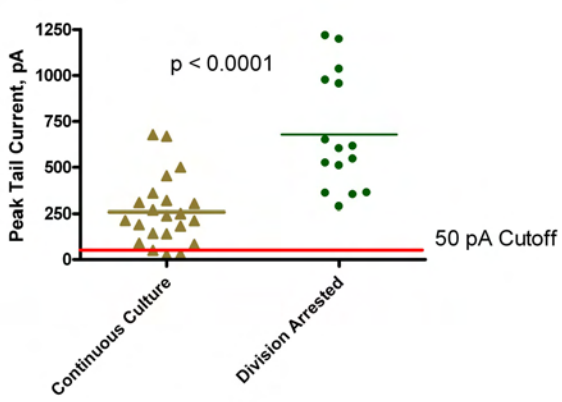

Figure 2. Example of currents recorded from dividing and division-arrested hERG-T-REx™ 293 cells in patch-clamp assay. The cell membrane potential was held at -90 mV, stepped briefly to -70 mV, then to +40 mV and then back to -70 mV to elicit tail currents. The mean peak tail current for the division-arrested cells (filled circles) was significantly larger by one-tailed t-test than for cells dividing in continuous culture (filled triangles).

2.0 OVERVIEW OF HERG-T-REX™ 293 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.

3.0 MATERIALS SUPPLIED

Product:	Name	Size	Catalog #
	hERG-T-REx™ 293 DA cells Contains sufficient division-arrested cells to assay one typical high-throughput-patch-clamp plate. Includes: • hERG-T-REx™ 293 DA cells (K1383) • Protocol • Certificate of Analysis	1 tube	K1383
	hERG-T-REx™ 293 DA cells Contains sufficient division-arrested cells to assay five typical high-throughput-patch-clamp plate. Includes: • hERG-T-REx™ 293 DA cells (K1384) • Protocol • Certificate of Analysis	1 tube	K1384
	hERG-T-REx™ 293 cells Includes: • ~2,000,000 (2×10^6 cells/ml) hERG-T-REx™ 293 cells (K1236) • Protocol • Certificate of Analysis	1 tube	K1236
Shipping Condition:	Dry Ice		
Storage Condition of Cells:	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.**		
Growth Properties of Non-Division-arrested Cells:	Adherent		
Cell Phenotype:	Epithelial		
Selection Marker(s) for Non-Division arrested cells:	Blasticidin 5 µg/ml; Geneticin® 400 µg/ml;		
***Mycoplasma* Testing:**	Negative		
BioSafety Level:	2		

4.0 MATERIALS REQUIRED

Use the table below to determine the additional media and reagents required:

Media/Reagents	Recommended Source	Part #
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648-010
DMEM (high-glucose), 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 (antibiotics)	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	25300-054
Blasticidin	Invitrogen	R210-01
Geneticin®	Invitrogen	10131-027
Doxycycline hydrochloride	MP Biomedicals	195044
Trypan Blue	Invitrogen	15250-061

The following table lists additional items required for use in manual patch-clamp assays:

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

5.0 DETAILED CELL HANDLING PROCEDURES

Note: Division-arrested (DA) cells have different thawing procedures than dividing cells. Refer to the instructions below for your particular application.

Note: Refer to **Section 6.0, Media Requirements** for specific media recipes.

5.1 DA Cells Thawing Method

Note: Once cells are thawed per the instructions below, cells must be counted and the density adjusted to the appropriate level as specified in **Section 7.0, Assay Procedure**, or similar, prior to analysis.

1. Rapidly thaw the vial of cells by placing at 37°C in a water bath with gentle agitation for 1–2 minutes. Do not submerge vial in water.
2. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
3. Transfer the vial contents into 10 ml of growth medium, PBS or suitable recording solution in a sterile 15-ml conical tube.
4. Centrifuge cells at 200 × *g* for 5 minutes to pellet.
5. Aspirate supernatant and resuspend the cells in fresh growth medium, PBS or suitable recording solution.
6. Count the cells.

7. Adjust the cell density with growth medium, PBS or suitable recording solution to the appropriate cell density as specified in Section 7.0. or your protocol. Proceed to **Section 7.0, Assay Procedure**, for guidance on using cells in an assay.

5.2 Dividing Cells

5.2.1 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 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.

5.2.2 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.

5.2.3 Freezing Method

1. Harvest the cells as described in **Section 5.2.2**. After detachment, count the cells, then spin cells down and resuspend in 4°C Cell Culture Freezing Medium to 2×10^6 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.

6.0 MEDIA REQUIREMENTS

6.1 Media Required

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

Note: Make **NO MEDIA SUBSTITUTIONS**, as these cell lines have been specifically validated for optimal assay performance with these media. For dividing cells, we recommend that you create and store an aliquot for back up.

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)	5 µg/ml	—
Geneticin (antibiotic)	400 µg/ml	--
Recovery™ Cell Culture Freezing Medium	—	100%

7.0 ASSAY PROCEDURE

The following instructions outline the general procedure for using hERG-T-REx™ 293 DA or hERG-T-REx™ 293 cells in manual patch-clamp assays to measure current block. Cells are plated onto suitable coverslips at 2–4 × 10^5 cells per 47 mm^2 dish and induced with doxycycline at 1 µg/ml. Dividing hERG-T-REx™ 293 cells require induction 48 hours before the assay. The hERG-T-REx™ 293 DA cells can be used in patch-clamp assays directly after thawing and at least the following day. 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.

7.1 Detailed Assay Protocol

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

7.1.1 Precautions

Note: Employ good patch-clamp assay methods to obtain high-quality, high-resistance seals and whole-cell access and stable recording conditions.

7.1.2 Harvest and plate cells (dividing cells only)

1. Remove media and 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 count on a hemacytometer.
5. Dilute with medium to desired density (2–4 × 10^5 cells per 3 ml).
6. Add diluted cells to 47 mm^2 dish containing 8–10 5-mm round coverslips.
7. Return 47 mm^2 dish to TC incubator; allow cells to adhere to coverslips overnight.

7.1.3 Prepare Stock Solutions

Prepare compound test solutions as required.

7.1.4 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 GΩ 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 seconds, returning to the command potential to -70 mV for 2 seconds and then returning to the holding potential of -90 mV, every 5 seconds.
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.

8.0 REFERENCES

1. 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

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Use of Genetically Modified Organisms (GMO)

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