Data Sheet

hERG1a/1b Inducible HEK293 Cell Line

Immortalized Cell Line

SCC295

Pack Size ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

HERG channel inhibition assays for preclinical cardiac safety screening of new drug compounds use electrophysiological methodology and employ hERG-expressing recombinant cell lines. Despite evidence that the cardiac IKr potassium current is produced by heteromeric hERG1a/1b channels,[1, 2, 3] drug screening assays have long relied on homomeric hERG1a alone, because of the instability of hERG 1b expression in so-called "stable" recombinant cell lines.[4] The novel inducible hERG1a/1b heteromeric cell line offers an improved tool for preclinical cardiac toxicity screening, by enabling more accurate predictions of off-target drug effects on cardiac IKr, particularly in computational models relying on accurate IC50 measurements. hERG1a/1b cells also can facilitate the study of heteromeric assembly of the hERG 1a and 1b subunits during biogenesis.[5]

Source

The heteromeric hERG1a/1b Inducible HEK293 cell line, in which hERG1b protein expression is tightly controlled by doxycyclin dose and treatment time, was derived from a homomeric hERG 1a expressing HEK293 cell line.[5]

Short Tandem Repeat (STR Profile)

D3S1358:	15, 17	D7S820	11, 12	FGA:	23
TH01:	7, 9.3	D16S539:	9, 13	Amel:	X
D21S11:	28, 30.2	CSF1PO:	11, 12		
D18S51:	17, 18	Penta D:	9, 10		
Penta E:	7, 15	vWA:	16, 19		
D5S818:	8, 9	D8S1179:	12, 14		
D13S317:	12	TPOX:	11		

hERG1a/1b Inducible HEK293 cells are genetically modified.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from mouse, rat, Chinese hamster, Golden Syrian hamster, and Non-human Primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

hERG1a/1b Inducible HEK293 cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Representative Data

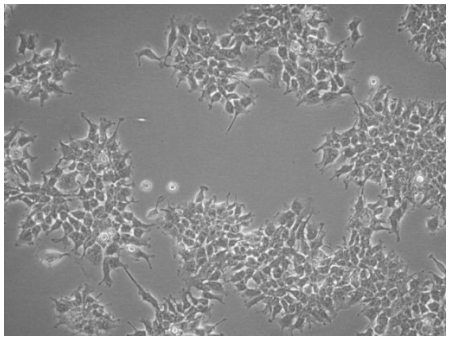

Figure 1. Bright-field images of hERG1a/1b Inducible HEK293 cells in culture, two days after thaw.

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in hERG1a/1b Inducible HEK293 Expansion Medium comprising High Glucose DMEM (Cat. No. SLM-120-B) with 10% FBS (Cat. No. ES-009-B), and selection antibiotic 5 mg/mL Geneticin (Cat. No. G8168), and 0.25 mg/mL Puromycin.

2. Remove the vial of frozen hERG1a/1b Inducible HEK293 cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.

3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of hERG1a/1b Inducible HEK293 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.

6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.

7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of hERG1a/1b Inducible HEK293 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. hERG1a/1b Inducible HEK293 cells should be passaged at ~ 80-85% confluency. Do not allow the cells to grow over 85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of hERG1a/1b Inducible HEK293 cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase® and incubate in a 37 °C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of hERG1a/1b Inducible HEK293 Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of hERG1a/1b Inducible HEK293 Expansion Medium to the conical tube and resuspend the cells thoroughly.

    IMPORTANT: Do not vortex the cells.

11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

hERG 1b Induction

Add doxycycline to hERG1a/1b Inducible HEK293 Expansion Medium at desired concentration; suggested range 100-200 ng/mL.

Cryopreservation of Cells

hERG1a/1b Inducible HEK293 cells may be frozen in hERG1a/1b Inducible HEK293 Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. *J Biol Chem* 2004, 279(43): 44690–44694.
2. *Circ Res* 2008, 103(7): e81–e95.
3. *PNAS* 2014, 111(50): 18073-18077.
4. *Br J Pharmacol* 2011, 164(2b): 419-432.
5. *Biophysical J* 2018, Volume 114, Issue 3, Suppl 1, 2 (292a) (Poster Abstract).

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