

Electroporation of Human Induced Pluripotent Stem (iPS) Cells with Ingenio® Electroporation Solution

Instructions for use with MIR 50111, 50112, 50114, 50115, 50117, 50118



SPECIFICATIONS

Storage	Store the Ingenio® Electroporation Solution at 4°C.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

► HUMAN iPSC ELECTROPORATION PROTOCOL USING AMAXA® NUCLEOFECTOR® II

NOTE: Prior to electroporation, grow iPS cells to ~80% confluence in mTeSR™1 medium. The following protocol describes the recommended electroporation conditions for cells grown in a 6-well plate.

Day 0: Electroporation of human iPS cells using Ingenio® Electroporation Solution

1. Verify that iPS cells appear healthy and have reached approximately 80% confluence.
2. Aspirate mTeSR™1 from wells and carefully wash cells once with 2 ml/well phosphate-buffered saline (PBS) without Ca^{2+} /Mg $^{2+}$.
3. To harvest iPS cells, add 1 ml Accutase™ per well and incubate at 37°C for 8-10 minutes.
4. Add 2 ml mTeSR™1 + ROCK inhibitor per well and pipet gently to break up cell clumps.
For Y-27632 ROCK Inhibitor: Use at 10 μM final concentration.
For Thiazoviven ROCK Inhibitor: Use at 2 μM final concentration.
5. Transfer cell suspension to sterile 50 ml conical tube. Centrifuge at 1000 rpm for 5 minutes.
6. Carefully aspirate the supernatant. Resuspend cell pellet in 1 ml/well mTeSR™1 + ROCK inhibitor.
7. Count cells using a cell counter or hemocytometer to determine concentration (cells/ml).
8. Transfer a volume containing 3×10^6 cells to a sterile 15 ml conical tube, and centrifuge cells at 1000 rpm for 5 minutes.
9. Carefully aspirate the supernatant. Resuspend the cell pellet in 100 μl Ingenio® Electroporation Solution.
10. Add 2-5 μg of concentrated, endotoxin-free nucleic acids (e.g. plasmid DNA, mRNA) to 100 μl cell + Ingenio® suspension. NOTE: The added volume of nucleic acid should be 20 μl or less.
11. Transfer cell mixture + nucleic acids to a 0.2 cm Ingenio® Electroporation Cuvette.
12. Use the Amaxa® Nucleofector® II program **B-016** for electroporation of cells.
13. Using a transfer pipette, immediately add 500 μl pre-warmed mTeSR™1 + ROCK inhibitor to the cuvette containing electroporated iPS cells.
14. Transfer cells to a 15 ml conical tube containing 12 ml pre-warmed mTeSR™1 + ROCK inhibitor. Mix gently by pipetting.
15. Plate 2 ml cell suspension per well into a Matrigel® coated 6-well plate.
16. Incubate cells overnight at 37°C, 5% CO $_2$.

Day 1: Media addition

1. Observe cells under a microscope to monitor health and viability.
2. Add 2 ml/well fresh TeSR™1 + ROCK inhibitor. DO NOT remove existing media from wells.

Day 2+: Media exchange and analysis of electroporated iPS cells

1. Aspirate media from wells and replace with 2 ml fresh mTeSR™1 *without* ROCK inhibitor.
2. Change medium daily and monitor growth of electroporated iPS cell colonies.
Optional: If required, add selection beginning 3-5 days post-electroporation. Maintain one well as an unselected (no antibiotic) control to monitor and compare cytotoxicity effects.
3. Pick colonies for screening and expansion approximately 2 weeks after electroporation.
4. If iPS cells were electroporated for genomic editing, colonies can be screened for integrants via PCR and clones can be analyzed by Southern blotting for off-target integration.

► TRANSFECTION NOTES

Reference Catalog Numbers:

Ingenio Electroporation Solution (Mirus Bio, MIR 50111, 50112, 50114, 50115, 50117, 50118)

Matrigel™ (Corning, 356234)

mTeSR™1 (Stem Cell Technologies, 05850)

Y-27632 ROCK Inhibitor (Stem Cell Technologies, 72302)

Thiazovivin ROCK Inhibitor (Stem Cell Technologies, 72252)

Accutase™ (Stem Cell Technologies, 07920)



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