

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 $\text{Ca}^{2+}/\text{Mg}^{2+}$.
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)



Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at: [mirusbio.com/ra](https://www.mirusbio.com/ra)

©1996-2024 All rights reserved. Mirus Bio LLC. All trademarks are the property of their respective owners.

For terms and conditions, visit www.mirusbio.com. For terms and conditions, visit www.mirusbio.com.

Rev.A 051817

Mirus Bio LLC

www.mirusbio.com | techsupport@mirusbio.com | Toll Free (U.S.): 844.MIRUSBIO | Direct: +1.608.441.2852