*Reverse Transfection of Human Induced Pluripotent Stem (iPS) Cells with Trans*IT®-LT1 Transfection Reagent

Instructions for use with *MIR 2300, 2304, 2305, 2306, 2310*

Mirus

SPECIFICATIONS

Storage	Store *Trans*IT®-LT1 Reagent tightly capped at 4°C. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

REVERSE PLASMID DNA TRANSFECTION PROTOCOL

NOTE: The following protocol describes a reverse transfection in a 6-well plate format.

Day 0: Reverse transfection of human iPS cells

1. Warm Matrigel™ coated plate for 30 minutes at room temperature.
2. Aspirate Matrigel™ from wells and add 0.5 ml of mTeSR™1 + ROCK inhibitor per well.
   For Y-27632 ROCK Inhibitor: Use at 10 µM final concentration.
   For Thiazoviven ROCK Inhibitor: Use at 2 µM final concentration.
3. Warm *Trans*IT®-LT1 Reagent to room temperature and vortex gently.
4. Place 400 µl OptiMEM® I Reduced-Serum Medium in a sterile tube.
5. Add 4 µg plasmid DNA to tube. Mix gently by pipetting.
6. Add 12 µl *Trans*IT®-LT1 Reagent. Mix gently by pipetting.
7. Incubate transfection complexes at room temperature for 15-20 minutes.
8. **While complexes are incubating**, begin iPS cell harvest by adding 1 ml Accutase™ per well (for cells in 6-well plate). Incubate for 10 minutes at 37°C.
9. Add 1ml mTeSR™1 + ROCK inhibitor per well. Pipette gently to break up cell clumps.
10. Transfer cell suspension to sterile 50 ml conical tube. Centrifuge at 1000 rpm for 5 minutes.
11. **While cells are being centrifuged**, add *Trans*IT®-LT1:DNA complexes (prepared in steps 3-7) to wells containing 0.5 ml mTeSR™1 + ROCK inhibitor (prepared in steps 1-2). Incubate at room temperature for the remainder of the cell preparation process.
    NOTE: The remaining incubation time should not exceed 15-20 minutes.
12. Once cells (from step 10) have completed centrifugation, carefully aspirate supernatant and resuspend cells in 10 ml mTeSR™1 + ROCK inhibitor.
13. Count cells with a cell counter or hemocytometer.
14. Centrifuge cells at 1000 rpm for 5 minutes. Resuspend cell pellet in mTeSR™1 + ROCK inhibitor at a final concentration of 1.2-2 x 10^6 cells per ml.
15. Plate 1 ml cell suspension per well containing *Trans*IT®-LT1:DNA complexes.
16. Incubate cells + transfection complexes overnight at 37°C, 5% CO_2.

Day 1: Media replacement

1. Aspirate media containing ROCK inhibitor and transfection complexes from wells and replace with 2 ml fresh mTeSR™1 *without* ROCK inhibitor.

Day 2-3: Transfection analysis of human iPS cells via flow cytometry (if applicable)

1. If the plasmid used for transfection encoded a fluorescent reporter, image cells using a fluorescent microscope.
2. To harvest cells, add 1 ml per well of TrypLE™ and incubate at 37°C for 5 minutes.
3. Add 1 ml mTeSR™1 + ROCK inhibitor per well. Pipet gently to break up cell clumps.
4. Transfer cell suspension to a sterile conical tube. Centrifuge at 1000 rpm for 5 minutes.
5. Resuspend cell pellet in 1 ml of mTeSR™1 + ROCK inhibitor.
6. Count cells using a cell counter or hemocytometer.
7. Perform flow cytometry using the remaining cells to determine transfection efficiency.
   NOTE: Use cells mock-transfected with reagent alone (no DNA) as a negative control.

▸ TRANSFECTION NOTES

Reference Catalog Numbers:

*Trans*IT®-LT1 (Mirus Bio, MIR 2300)
Matrigel™ (Corning, 356234)
mTeSR™1 (STEMCELL Technologies, 05850)
Y-27632 ROCK Inhibitor (STEMCELL Technologies, 72302)
Thiazovivin ROCK Inhibitor (STEMCELL Technologies, 72252)
Accutase™ (STEMCELL Technologies, 07920)
Opti-MEM®I Reduced-Serum Medium (Life Technologies, 31985-062)
TrypLE™ (Life Technologies, A12177-01)

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.

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