Transfection Protocol for THP-1 cells (ATCC)

Keywords

Neon, Transfection, Protocol, Microporator, Electroporation, MP-100

CellName

THP-1

Payload

plasmid

Protocol Description

The Neon™ Transfection System is a novel, benchtop electroporation device that employs a pipette tip as an electroporation chamber to efficiently transfect mammalian cells, including primary cells. THP-1 cells are suspension monocytes first isolated from the peripheral blood of a patient with acute monocytic leukemia. THP-1 cells express Fc and C3b receptors but lack both surface and cytoplasmic immunoglobulins. THP-1 cells also stain positive for alpha-naphthyl butyrate esterase. They produce lysozymes and are phagocytic. THP-1 cells can also restore the response of T lymphocytes to lectin protein Con A. THP-1 cells are a good model for the study of monocyte-macrophage differentiation, which can be induced with the phorbol esters (PMA) or vitamin D3. THP-1 cells are also widely used for the study of ERK, p38 MAPK and JNK signaling pathways, as well as cytokine receptor-mediated STAT signaling pathway. The following protocol provides instructions for using the Neon™ Transfection device to transfect THP-1 cells with plasmid DNA. The best electroporation conditions resulted in 44% transfection efficiency with 51% viability.

Reagents & Equipment

THP-1 cells (ATCC® Cat. no. TIB-202™) RPMI 1640 medium (Cat. No. 11875) 2-mercaptoethanol (1000X), lipid (Cat No. 21985-023) Fetal Bovine Serum, Certified, (Cat. no. 16000-036) Plasmid DNA of interest (concentration should be at least 0.5 µg/mL in deionized water or TE) Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid without Ca2+ and Mg2+ (Cat. no. 14190-144) Neon™ Transfection system (Cat. no. MPK5000) Neon™ Kit, 10 µL (Cat. no. MPK1096) or Neon™ Kit, 100 µL (Cat. no. MPK10096) Appropriate tissue culture plates and supplies such as centrifuge tubes, pipettes, Pasteur pipettes, Eppendorf tubes, tips, etc.

Procedure

Media Preparation:

RPMI Complete Growth Media: RPMI-1640, 0.05 mM 2-mercaptoethanol, 10% FBS, Filter Sterilized

Work inside Biosafety Hood. Add components to the 500 mL Filter Flask.

500 mL RPMI-1640

50 mL Fetal Bovine Serum, Certified

454 uL of 2-mercaptoethanol (55 mM in DPBS)

Culturing THP-1 cells

Thawing Protocol

Thaw THP-1 cells at least 7 days prior and passage two times before plating for transfection. For example: Thaw cells on Friday, and passage on the following Monday and Friday.

1. Pre-warm 50 mL conical tube containing 30 mL RPMI Complete Growth Media to room temperature
2. Remove vial of THP-1 cells from LN_2 tank, 6-10 x 10^6 viable cells. Twist cap to open and close to release excess pressure.
3. Thaw in 37 °C water bath until 90% thawed, approximately 2-4 minutes.
4. Remove from water bath and wipe with 70% ethanol. Slowly add the content to 9 mL pre-warmed RPMI Complete Media. Spin at 125 xg (about 800 rpm) for 7 min. Aspirate away the medium. Resuspend cell pellet in 10 mL pre-warmed RPMI Complete Media.
5. Take 1 mL to count cells with Via-Cell using Default protocol. Record density and viability.
6. Seed the cells into T 75 flask for a density of ~0.4-0.6 x 10^4 cells per mL based on 6-10 x 10^6 viable cells

Subculturing and Expansion

1. Transfer floating cells by pipetting to a 50 mL conical tube.
2. Centrifuge and resuspend the pellet in fresh medium.
3. Determine the total number of cells and percent viability.
4. From the resulting suspension dilute cells to a concentration 2-4 X 10^5 cells/ml into fresh medium in a new flask. Subcultutre when cell concentration reaches 8x10^5 cells/ml. Keep culture below approximate density of 1 x 10^6 cells/ml.
5. Place cells in incubator at 37 °C, 5% CO_2, 100% relative humidity

Medium Renewal **:** Every 2 to 3 days

Based on the number of cells needed on the day of transfection, expand the cells accordingly (taking into account that cell doubling time is approximately 40-50 hours).

Three days prior to electroporation: seed cells at 0.2x10 ^{6} cells/ml

Four days prior to electroporation: seed cells at 0.15x 10 ^{6}cells/ml

On the day of electroporation, the cell density should be around 0.8-1x10 ^{6}/ml, with >95% viability.

Neon™ Transfection Protocol

Use this procedure to transfect plasmid DNA into THP-1 cells in a 24-well format (for other plate formats, please refer to Neon Manual ™ or Quick Reference Guide for scaling guidlines). Based on your initial results, you may need to further optimize the electroporation parameters for your experiment.

1. Cultivate the required number of cells (see table below for cell amount) such that the cells are 70–90% confluent on the day of the experiment.
2. On the day of the experiment, harvest and wash the cells in phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}.
3. Resuspend the cell pellet in Resuspension Buffer R (included with Neon™ Kits) at a final concentration of 2.0×10^{7} cells/mL. Prepare enough cells for at least five transfections (ie, 1.0 x 10 ^{6} cells in 50 µL)
4. Transfer the cells to a sterile, 1.5 mL microcentrifuge tube.
5. Prepare 24-well plates by filling the appropriate number of wells with 0.5 mL of RPMI Complete Growth Media without antibiotics and pre-incubate the plates at 37°C in a humidified 5% CO_2 incubator. If using other plate formats, adjust the volume accordingly.
6. Turn on the Neon™ unit and enter the electroporation parameters in the Input window (see table below).

Electroporation parameters

Voltage (V): 1400 Pulse Width (ms): 20 Pulse Number: 2 Cells/ml: 2X 10 ^{7} Tip Type: 10ul

Voltage (V): 1700 Pulse Width (ms): 20 Pulse Number: 1 Cells/ml: 2X 10 ^{7} Tip Type: 10ul

7. Fill the Neon™ Tube with 3 mL Electrolytic Buffer (use Buffer E for the 10 µL Neon™ Tip and Buffer E2 for the 100 µL Neon™ Tip).
8. Insert the Neon™ Tube into the Neon™ Pipette Station until you hear a click sound.
9. Transfer 1.0 µg plasmid DNA per transfection reaction (i.e. 5.0 µg for 5 transfections) to the tube containing cells and mix gently.

10. Insert a Neon™ Tip into the Neon™ Pipette.
11. Press the push-button on the Neon™ Pipette to the first stop and immerse the Neon™ Tip into the cell-DNA mixture. Slowly release the push-button on the pipette to aspirate the cell-DNA mixture into the Neon™ Tip.
12. Insert the Neon™ Pipette with the sample vertically into the Neon™ Tube placed in the Neon™ Pipette Station until you hear a click sound.
13. Ensure that you have entered the appropriate electroporation parameters and press Start on the Neon™ touchscreen to deliver the electric pulse.
14. The touchscreen displays **“Complete”** to indicate that electroporation is complete.
15. Remove the Neon™ Pipette from the Neon™ Pipette Station and immediately transfer the samples from the Neon™ Tip into the prepared culture plate containing pre-warmed RPMI complete growth medium without antibiotics. Discard the Neon™ Tip into an appropriate biological hazardous waste container.
16. Repeat Steps 10–15 for the remaining samples. Use tips no more than twice.
17. Gently rock the plate to assure even distribution of the cells. Incubate the plate at 37°C in a humidified CO2 incubator.
18. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay).

Results

Voltage (V): 1400 Pulse Width (ms): 20 Pulse Number: 2 Cells/ml: 2X 107 Tip Type: 10ul Transfection efficiency: 41% Viability 47% Voltage (V): 1700 Pulse Width (ms): 20 Pulse Number: 1 Cells/ml: 2X 107 Tip Type: 10ul Transfection efficiency: 44% Viability 51%

Attachments

THP-1_fluorescent uptake.pdf

Fluorescent uptake in THP-1 cells

References

Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K (1980). Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int. J. Cancer 26 (2): 171–6.

Link to Protocol Online

http://protocolexchange.community.invitrogen.com/protocol/1092