Episomal iPSC Reprogramming Vectors

Part Number A14702PIS **Publication Number** MAN0006963 **Revision Date** 7 June 2012

Description

Episomal iPSC Reprogramming Vectors are a non-integrating system that reprograms somatic cells into induced pluripotent stem cells (iPSCs) in a feeder-free environment. Each tube of Episomal iPSC Reprogramming Vectors provides an optimized mixture of three oriP/EBNA-1 (Epstein-Barr nuclear antigen-1) episomal vectors containing the six reprogramming factors (Oct4, Sox2, Nanog, Lin28, Klf4, and LMyc). High transfection efficiency due to oriP/EBNA-1 mediated nuclear import and retention of vector DNA allows iPSC derivation in a single transfection (Yu *et al.*, 2011). Silencing of the viral promoter driving EBNA-1 expression and the loss of the episomes at a rate of ~5% per cell cycle due to defects in vector synthesis and partitioning allows the removal of episomal vectors from the iPSCs without any additional manipulation (Nanbo *et al.*, 2007).

Kit Name	Catalog no.	Amount	Storage
Episomal iPSC Reprogramming Vectors	A14703	50 µL (1 µg/µL)	Store at –15°C to –25°C

Product Use

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

Guidelines for Reprogramming Fibroblasts

- Each reprogramming experiment requires 1×10^6 cells and 8.5 µL of Episomal iPSC Reprogramming Vectors. Each tube of Episomal iPSC Reprogramming Vectors contains sufficient material for 5 reprogramming experiments.
- Cells should be approximately 75–90% confluent on the day of transfection (Day 0). Since overconfluency results in decreased transfection efficiency, we recommend replating the cells to achieve 75–90% confluency, if they have become overconfluent during culturing.
- We recommend reprogramming patient cells at the earliest passage possible. However, it is important to have the cells growing and healthy, which can take between 1–4 weeks. The cells are usually ready to reprogram once they have gone through a total of 3–4 passages.
- Transfect the cells by electroporation and then plate the transfected cells onto vitronectin-coated culture dishes. We recommend using the Neon® Transfection System (Cat. no. MPK5000) for electroporation.
- Culture the cells in Fibroblast Medium (unsupplemented) until the day of transfection. For recovery after transfection, culture the cells in Supplemented Fibroblast Medium overnight, and then switch to N2B27 Medium supplemented with CHALP molecule cocktail and bFGF. After 15 days of culture, switch to Essential 8™ Medium.

  Note: CHALP molecule cocktail is an optimized mixture of small molecules (**C**HIR99021, **H**A-100, **A**-83-01, h**L**IF, **P**D0325901) shown to greatly improve the episomal reprogramming efficiency.
- You do not need to manipulate the culture conditions to remove the reprogramming vectors. In the absence of drug selection, approximately 5% of the episomal vectors are lost each cell cycle due to silencing of the viral promoter driving EBNA-1 expression and defects in vector synthesis and partitioning.
- Newly derived PSC lines may contain a fair amount of differentiation through passage 4. It is not necessary to remove differentiated material prior to passaging. By propagating/splitting the cells the overall culture health should improve throughout the early passages.

Workflow

A typical reprogramming schedule using the Episomal iPSC Reprogramming Vectors is shown below. For more information and detailed protocols, refer to the Episomal iPSC Reprogramming Vectors User Guide, which is available for downloading at **www.lifetechnologies.com/manuals**.

Day –4 to –2: Plate human fibroblasts into a T75 flask in Fibroblast Medium so that they are 75–90% confluent on the day of transfection (Day 0).

Day 0: Transfect the cells using the Neon® Transfection System. Plate transfected cells onto vitronectin-coated culture dishes and incubate overnight in Supplemented Fibroblast Medium.

Day 1 to 14: Change the medium to N2B27 Medium supplemented with CHALP molecule cocktail and bFGF; replace the spent medium every other day.

Day 15: Change the medium to Essential 8™ Medium and monitor the culture vessels for the emergence of iPSC colonies.

Day 21: Pick and transfer undifferentiated iPSCs onto fresh vitronectin-coated culture dishes for expansion.

Preparing Media

Fibroblast Medium and Supplemented Fibroblast Medium

1. For 100 mL of Fibroblast Medium, aseptically mix the following components. Fibroblast Medium can be stored at 2°C to 8°C for up to 1 week.

Component	Amount
DMEM High Glucose with GlutaMAX™-I and Pyruvate	89 mL
MEM Non-essential Amino Acids Solution (10 mM)	1 mL
Fetal Bovine Serum (FBS), ESC-Qualified	10 mL

2. For Supplemented Fibroblast Medium, add the following components to Fibroblast Medium **freshly, just prior to use**.

   Note: You will need 30 mL of Supplemented Fibroblast Medium per transfection.

Component	Final conc.
Basic Fibroblast Growth Factor (bFGF)	4 ng/mL
HA-100 (ROCk inhibitor)	10 µM

N2B27 Medium Supplemented with CHALP Molecules and bFGF

1. For 250 mL of N2B27 Medium, aseptically mix the following components. N2B27 Medium can be stored at 2°C to 8°C for up to 1 week.

Component	Amount
DMEM/F-12 with HEPES	238.75 mL
N-2 Supplement (100X)	2.5 mL
B-27® Supplement (50X)	5.0 mL
MEM Non-essential Amino Acids Solution (10 mM)	2.5 mL
GlutaMAX™-I (100X)	1.25 mL
β-Mercaptoethanol (1000X)	454.5 µL

2. To supplement N2B27 Medium with the CHALP molecule cocktail and bFGF, add the following components **freshly, just prior to use**.

Component	Final conc.
PD0325901 (MEK inhibitor)	0.5 µM
CHIR99021 (GSK3β inhibitor)	3 µM
hLIF (Human Leukemia Inhibitory Factor)	10 ng/mL
A-83-01 (TGF-β/Activin/Nodal receptor inhibitor)	0.5 µM
HA-100 (ROCk inhibitor)	10 µM
Basic Fibroblast Growth Factor (bFGF)	100 ng/mL

Note: CHALP molecule cocktail is an optimized mixture of small molecules (**C**HIR99021, **H**A-100, **A**-83-01, h**L**IF, **P**D0325901) shown to greatly improve the episomal reprogramming efficiency.

Essential 8™ Medium

1. Thaw the frozen Essential 8™ Supplement at 2°C to 8°C overnight. **Do not thaw the frozen supplement at 37°C.**
2. Mix the thawed supplement by gently inverting the vial a couple of times, remove 10 mL from the bottle of DMEM/F-12 (HAM) 1:1, and then aseptically transfer the entire contents of the Essential 8™ Supplement to the bottle of DMEM/F-12 (HAM) 1:1. Swirl the bottle to mix and to obtain 500 mL of homogenous complete medium.
3. Complete Essential 8™ Medium can be stored at 2°C to 8°C for up to 2 weeks. Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch. **Do not warm the medium at 37°C.**

Coating Culture Vessels with Vitronectin

Instructions for coating a 100-mm culture dish with vitronectin (VTN-N) at a coating concentration of 0.5 µg/cm^2 are provided below. Prepare two vitronectin-coated 100-mm dishes for each transfection. For coating other culture vessels or using other coating concentrations, refer to the instructions provided with vitronectin.

1. Remove a 60-µL aliquot of vitronectin from –80°C storage and thaw at room temperature. You will need one 60-µL aliquot per 100-mm dish.
2. Add 60 µL of thawed vitronectin into a 15-mL conical tube containing 6 mL of sterile DPBS without Calcium and Magnesium (Cat. no. 14190-144) at room temperature. Gently resuspend by pipetting the vitronectin dilution up and down.
3. Add the diluted vitronectin solution to a 100-mm culture dish and incubate at room temperature for 1 hour.

   Note: The culture vessel can be used immediately or stored at 2–8°C wrapped in laboratory film for up to a week. Do not allow the vessel to dry.
4. Prior to use, aspirate the vitronectin solution and discard. It is not necessary to rinse off the culture vessel after the removal of vitronectin.

Reprogramming Fibroblasts

The following protocols have been optimized for human neonatal foreskin fibroblast cells (strain BJ; ATCC no. CRL2522). We recommend that you optimize the protocol for your cell type.

Day –4 to –2:

Seed Cells

1. Four to two days before transfection, plate human fibroblast cells in Fibroblast Medium into a T75 flask so that they are 75–90% confluent on the day of transfection (Day 0).

   Note: Growth rate is dependent on the cell line and culture conditions. Depending on the seeding density and culture conditions, the cells may take up to 5 days to reach 75–90% confluency..

Day 0:

Prepare Cells for Transfection

1. Add 6 mL of Supplemented Fibroblast Medium to a 15-mL conical tube for each transfection (1 tube per transfection). Incubate the tubes at 37°C until needed (step 22).
2. Aspirate the vitronectin solution from vitronectin-coated dishes and replace with 12 mL of fresh Supplemented Fibroblast Medium. Place the coated dishes at 37°C until ready for use.

   Note: You will need two 100-mm vitronectin-coated dishes for each transfection.
3. Aspirate the spent medium from fibroblasts in T75 flasks.
4. Wash the cells in DPBS without Calcium and Magnesium.
5. Add 2 mL of 0.05% Trypsin/EDTA to each flask.
6. Incubate the flasks at 37°C for approximately 4 minutes.
7. Add 6 mL of Supplemented Fibroblast Medium to each flask. Tap the plate against your hand to dislodge the cells from the plate and carefully transfer the cells into an empty 15-mL conical tube.

   Note: Each T75 flask provides plenty of cells for transfection, so any residual cells still clinging to the flask after Trypsin/EDTA treatment may be left behind.
8. Remove a 20-µL sample to perform a viable cell count and calculate the number of transfections to be performed. You will need 1×10^6 cells per transfection.

   Number of transfections = Number of viable cells/(1×10^6)
9. Transfer enough cells for up to three transfections (i.e., 1×10^6 to 3×10^6 cells) into a new 15-mL conical tube.
10. Bring the volume to 10 mL in the new tube with Supplemented Fibroblast Medium and centrifuge cells at 1,000 rpm for 5 minutes at room temperature.
11. Carefully aspirate most of the supernatant using a glass Pasteur pipette, leaving approximately 100–200 µL behind. Remove the remaining medium with a 200-µL pipette.

Transfect Cells using the Neon® Transfection System

12. Resuspend the cell pellet in Resuspension Buffer R (included with Neon® Transfection kits) at a final concentration of 1.0×10^6 cells/0.1 mL.
13. Transfer the cells (100 µL per transfection reaction) to a sterile 1.5-mL microcentrifuge tube.
14. Turn on the Neon® unit and enter the following electroporation parameters in the Input window.

Pulse Voltage	Pulse Width	Pulse Number	Cell Density	Tip Type
1650 V	10 ms	3	1×10^6 cells/0.1 mL	100 µL

15. Fill the Neon® Tube with 3 mL Electrolytic Buffer (use Buffer E2 for the 100 µL Neon® Tip).
16. Insert the Neon® Tube into the Neon® Pipette Station until you hear a click.

17. Transfer 8.5 µL of Episomal iPSC Reprogramming Vectors per transfection reaction to the tube containing the cells and mix gently.
18. Insert a Neon® Tip into the Neon® Pipette.
19. 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.

    Note: Avoid air bubbles during pipetting to prevent arcing during electroporation. If you notice air bubbles in the tip, discard the sample and carefully aspirate fresh sample into the tip.
20. Insert the Neon® Pipette with the sample vertically into the Neon® Tube placed in the Neon® Pipette Station until you hear a click.
21. Ensure that you have entered the appropriate electroporation parameters and press **Start** on the Neon® touchscreen to deliver the electric pulse.
22. Remove the Neon® Pipette from the Neon® Pipette Station and immediately transfer the samples from the Neon® Tip into the 15-mL tube containing 6 mL of pre-warmed Supplemented Fibroblast Medium (prepared in step 1).
23. Mix the transfected cells by gentle inversion and pipette 3 mL into a 100-mm vitronectin-coated plate (two plates per transfection). Evenly distribute cells across plate. Discard the Neon® Tip into an appropriate biological hazardous waste container.
24. Repeat the process for any additional samples. Do not use a Neon® tip more than twice.
25. Incubate the plates at 37°C in a humidified CO_2 incubator overnight.

Day +1:

Switch to Supplemented N2B27 Medium

1. Aspirate the spent Supplemented Fibroblast Medium from the plates using a Pasteur pipette.
2. Add 10 mL of N2B27 Medium supplemented with CHALP molecule cocktail and bFGF (added fresh daily) to each 100-mm plate.
3. Replace the spent medium every other day, up to day 15 post-transfection.

Days +15:

Switch to Essential 8™ Medium

1. Aspirate the spent medium and replace with Essential 8™ Medium. Resume medium changes every other day.
2. Observe the plates every other day under a microscope for the emergence of cell clumps indicative of transformed cells Within 15 to 21 days of transfection, the iPSC colonies will grow to an appropriate size for transfer.

Identifying Fibroblasts

By day 21, the colonies on the plates will appear large and compact and cover the majority of the surface area of the culture vessel. However, only a fraction of these colonies will consist of iPSCs, which exhibit a hESC-like morphology characterized by a flatter cobblestone-like appearance with individual cells clearly demarcated from each other in the colonies. Therefore, we recommend that you perform surface marker staining with Tra1-60 or Tra1-81 antibodies that recognize undifferentiated iPSCs. For more information, refer to the Episomal iPSC Reprogramming Vectors User Guide, available at **www.lifetechnologies.com/manuals**.

Note: Other methods of identifying iPSCs (such as alkaline phosphatase staining) are also acceptable.

Picking iPSC Colonies

1. Examine the culture dish containing the reprogrammed cells under 10X magnification using an inverted microscope, and mark the colony to be picked on the bottom of the culture dish.

   Note: We recommend picking at least 10 distinct colonies by the end of each reprogramming experiment and expanding them in separate vitronectin-coated 24-well plates.
2. In a sterile cell culture hood (i.e., biosafety cabinet) equipped with a stereomicroscope, cut the colony to be picked into 5–6 pieces in a grid-like pattern using a 25-gauge 1½ inch needle.
3. Using a 200-µL pipette, transfer the cut pieces to a freshly prepared vitronectin-coated 24-well plate containing Essential 8™ Medium.
4. Incubate the vitronectin -coated plate containing the picked colonies in a 37°C, 5% CO_2 incubator.
5. Allow the colonies to attach to the culture plate for 48 hours before replacing the spent medium with fresh Essential 8™ Medium. After that, change the medium every day.

Passaging iPSCs

- Treat the reprogrammed iPSC colonies like normal human iPSC colonies; passage, expand, and maintain them using standard culture procedures until you have frozen cells from two 60-mm plates.
- Cells cultured in Essential 8™ Medium on vitronectin -coated culture vessels should be passaged using 0.5 mM EDTA in DPBS. Use of enzymes such as collagenase and dispase for passaging these cells results in compromised viability and attachment. For more information, refer to the Episomal iPSC Reprogramming Vectors User Guide or the Essential 8™ Medium (Prototype) User Guide, which are available for downloading at **www.lifetechnologies.com/manuals**.

Detecting Episomal Vectors by PCR

You can detect the presence of Episomal iPSC Reprogramming Vectors in reprogrammed iPSC colonies by endpoint PCR using the PCR primers listed below. For more information, refer to the Episomal iPSC Reprogramming Vectors User Guide available at **www.lifetechnologies.com/manuals**.

Target: oriP (544 bp expected size)

Primers	Sequence
pEP4-SF1-oriP	5'-TTC CAC GAG GGT AGT GAA CC-3'
pEP4-SR1-oriP	5'-TCG GGG GTG TTA GAG ACA AC-3'

Target: EBNA-1 (666 bp expected size)

Primers	Sequence
pEP4-SF2-oriP	5'-ATC GTC AAA GCT GCA CAC AG-3'
pEP4-SR2-oriP	5'-CCC AGG AGT CCC AGT AGT CA-3'

References

Nanbo, A., Sugden, A., and Sugden, B. (2007) The coupling of synthesis and partitioning of EBV's plasmid replicon is revealed in live cells. EMBO J *26*, 4252–4262.

Yu, J., Chau, K. F., Vodyanik, M. A., Jiang, J., and Jiang, Y. (2011) Efficient Feeder-Free Episomal Reprogramming with Small Molecules. PLoS One *6*, e17557.

Related Products

The following products used in episomal iPSC reprogramming are available from Life Technologies.

Product	Cat. no.
Dulbecco's Modified Eagle Medium (DMEM) with GlutaMAX™-I (High Glucose)	10569-010
Fetal Bovine Serum (FBS), ESC-Qualified	16141-079
MEM Non-Essential Amino Acids Solution, 10 mM	11140-050
Basic Fibroblast Growth Factor (bFGF)	PHG0264
Essential 8™ Medium (Prototype)	A14666SA
DMEM/F-12 with HEPES	11330-057
N-2 Supplement (100X)	17502-048
B-27® Supplement (50X)	17504-044
GlutaMAX™-I (100X)	35050-061
β-mercaptoethanol, 1000X	21985-023
Dulbecco's PBS (DPBS) without Calcium and Magnesium	14190-144
Vitronectin, truncated human recombinant (rhVTN-N)	A14701SA
0.05% Trypsin-EDTA (1X), Phenol Red	25300-054
UltraPure™ 0.5 M EDTA, pH 8.0	15575
Neon® Transfection System	MPK5000
LIF (Human Leukemia Inhibitory Factor)	PHC9461

The following products are available from other vendors.

Product	Supplier	Cat. no.
PD0325901 (MEK inhibitor)	Stemgent	04-0006
CHIR99021 (GSK3β inhibitor)	Stemgent	04-0004
A-83-01 (TGF-β/Activin/Nodal receptor inhibitor)	Stemgent	04-0014
HA-100 (ROCk inhibitor)	Santa Cruz	sc-203072

Documentation and Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at **www.lifetechnologies.com/gibco**. For further assistance, email our Technical Support team at **techsupport@lifetech.com**.

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

Expir. date | Storage condition | Read SDS | Research Use Only | Lot/batch # | Cat. #

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