Episomal iPSC Reprogramming Vectors
FAQs

About Episomal iPSC Reprogramming Vectors

Episomal iPSC Reprogramming Vectors – Catalog Number A14703

1. What are induced pluripotent stem cells (iPSCs)?

   iPSCs are genetically reprogrammed somatic cells which exhibit a pluripotent stem cell–like state similar to embryonic stem cells. iPSCs can be derived by inducing selected gene expression via various methods.

2. What are the Episomal iPSC Reprogramming Vectors?

   The Episomal iPSC Reprogramming Vectors are a non-integrating system that reprograms somatic cells into induced pluripotent stem cells (iPSCs). This product is a mixture of three vectors designed to provide the optimal system for generating transgene-free and virus-free iPSCs in a feeder-free environment. Originally developed by Junying Yu and James Thomson (1), and further optimized by Cellular Dynamics International, these Episomal iPSC Reprogramming Vectors have proven successful in reprogramming a number of different somatic cell types.

3. What vectors are included in the Episomal iPSC Reprogramming Vectors?

   The optimized mixture of three vectors has the oriP/EBNA-1 (Epstein-Barr nuclear antigen-1) backbone that delivers six reprogramming factors: Oct4, Sox2, Nanog, Lin28, Klf4 and L-Myc. The vectors also contain SV40LT.

4. How do the Episomal iPSC Reprogramming Vectors work?

   The episomal vectors are introduced into the cell by electroporation. These non-integrating vectors replicate only once per cell cycle, with activation of replication by binding of multiple EBNA-1 homodimers to oriP within the nucleus (2).

5. What are the advantages of using oriP/EBNA vectors?

   oriP/EBNA vectors have been successfully used to deliver the reprogramming genes. High transfection efficiency due to oriP/EBNA-1 mediated nuclear import and retention of vector DNA allows iPSC derivation in a single transfection (3). 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 (4).

6. What are the benefits of using a viral-free reprogramming method?

   The Episomal iPSC Reprogramming Vectors are a well-described system for producing transgene-free, virus-free iPSCs providing a source of iPSCs (2). In general, DNA vectors are more stable than viruses and can be conveniently thawed and re-frozen through several usages.

7. What are the benefits of using an integration-free reprogramming method?

   Integration-free reprogramming methods generate iPSCs that do not contain detectable vectors or transgenes. Traditional technologies used for reprogramming (e.g., lentivirus, retrovirus) integrate into the genome of the target cells. The resulting iPSCs and cells differentiated from those iPSCs will contain foreign DNA and could be unsafe and problematic for use in cell therapy and drug discovery applications. Furthermore, the integration could occur in a critical region of the genome, causing problems with unrelated developmental processes.

8. What cell types have been successfully reprogrammed with Episomal iPSC Reprogramming Vectors?

   iPSCs have been generated with episomal vectors from a range of somatic cells including fibroblasts, bone marrow mononuclear cells (5), Peripheral Blood Mononuclear Cells (PBMCs), and Lymphoblast B Cells and various disease type fibroblasts.

9. Can you reprogram mouse cells with this kit?

   We currently only have data with reprogramming human cells with this kit. However, successful reprogramming with mouse cells and episomal vectors has been demonstrated (6).

10. Are there safety concerns with Episomal iPSC Reprogramming Vectors?

    This product is safe for research purpose use only.

How to use the Episomal iPSC Reprogramming Vectors

11. How should I store the Episomal iPSC Reprogramming Vectors?

    Upon receipt, the kit should be stored at –15°C to –25°C.

12. Can Episomal iPSC Reprogramming Vectors go through multiple freeze/thaw cycles?

    The vectors can be frozen and re-thawed, however we recommend keeping freeze/thaw cycles to a minimum (2–3 cycles). The vectors should be kept at –15°C to –25°C for long term storage.

13. How many experiments can be performed with one kit?

    Each kit provides enough material for 5 reprogramming experiments. The total volume of vectors in each vial is 50 µL, and 8.5 µL are required per experiment.

14. How many cells do I need to start my reprogramming experiment with the Episomal iPSC Reprogramming Vectors?

For each reprogramming experiment, you will need 1×10^6 somatic cells to reprogram.

We recommend the following for fibroblasts, but this may vary with other cell types: Two to four days before transfection, plate cells into a T75 flask. Cells should be approximately 75–90% confluent on the day of transfection (Day 0). Depending on the seeding density and culture conditions, the cells may take up to 5 days to reach 75–90% confluency.

Since overconfluency results in decreased transfection efficiency, we recommend replating your cells to achieve 75–90% confluency if your cells have become overconfluent during culturing.

15. What is the optimal passage number for reprogramming patient fibroblasts?

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.

16. What is the recommended medium to use for my somatic cells?

This will vary based on the specific somatic cell type being used. For fibroblasts, we recommend the following:

To prepare 100 mL of complete medium, aseptically combine the components listed below:

Component	Stock Concentration	Final Concentration	Volume
Dulbecco's Modified Eagle Medium (DMEM), High Glucose with GlutaMax™-1 and Pyruvate (Cat. no. 10569-010)	—	1X	89 mL
MEM Non-Essential Amino Acids Solution (Cat. no. 11140-050)	10 mM	0.1 mM	1 mL
Fetal Bovine Serum (FBS), ESC-Qualified (Cat. no. 16141-079 or 10439-024)	—	10%	10 mL

17. How do I transfect the cells?

Cells must first be treated with trypsin to obtain a single cell suspension. $1x10^6$ cells are required for each reprogramming experiment. It is recommended to transfect the cells using the Neon® Transfection System (Cat. no. MPK5000), and then to plate the transfected cells onto two 100-mm vitronectin-coated dishes.

18. What is the expected transfection efficiency when using Neon® Transfection System?

Depending on the cell line/type, the expected transfection efficiency is between 0.02 and 0.001%.

19. **What is the expected percentage of cell death when transfecting with Neon® Transfection System?**

Cell death can be highly variable depending on cell type and cell health prior to transfection. We observe approximately 25% cell death post transfection with BJ fibroblasts in the recommended conditions.

20. **Once the cells are transfected, what medium should be used to plate the cells?**

Supplemented Fibroblast Medium should be used to plate the cells following transfection. On Day 1 post-transfection, the medium is changed to N2B27 Medium with CHALP molecule cocktail and bFGF. The recipes are given below:

To prepare 100 mL of **Supplemented Fibroblast Medium**, aseptically mix the following components:

Component	Stock Concentration	Final Concentration	Volume
Dulbecco's Modified Eagle Medium (DMEM), High Glucose with GlutaMax™-1 and Pyruvate (Cat. no. 10569-010)	—	1X	89 mL
MEM Non-Essential Amino Acids Solution (Cat. no. 11140-050)	10 mM	0.1 mM	1 mL
Fetal Bovine Serum (FBS), ESC-Qualified (Cat. no. 16141-079 or 10439-024)	—	10%	10 mL
Basic Fibroblast Growth Factor (bFGF) (Cat. no. PHG0264)*	10 µg/mL	4 ng/mL	40 µL
HA-100 (ROCk inhibitor) (Santa Cruz, Cat. no. sc-203072)*	varies	10 µM	varies

*Add freshly, just prior to use

To prepare 250 mL of **N2B27 Medium**, aseptically mix the following components:

Component	Stock Concentration	Final Concentration	Volume
DMEM/F-12 with HEPES (Cat. no. 11330-057)	—	1X	238.75 mL
N-2 Supplement (Cat. no. 17502-048)	100X	1X	2.5 mL
B-27® Supplement (Cat. no. 16141-079)	50X	1X	5.0 mL
MEM Non-Essential Amino Acids Solution (Cat. no. 11140-050)	10 mM	100 µM	2.5 mL
GlutaMAX™-I (Cat. no. 35050-061)	100X	0.5X	1.25 mL
β-mercaptoethanol (Cat. no. 21985-023)	1000X		454.5 µL

N2B27 Medium can be stored at 2–8°C for up to 1 week.

To supplement **N2B27 Medium with CHALP molecule cocktail and bFGF**, aseptically add the following components to N2B27 Medium to achieve the final concentrations shown below. **These must be added fresh, when the medium is used**:

Component	Final Concentration
PD0325901 (MEK Inhibitor) (Stemgent, Cat. no. 04-0006)	0.5 µM
CHIR99021 (GSK3β inhibitor) (Stemgent, Cat. no. 04-0004)	3 µM
A-83-01 (TGF-β/Activin/Nodal receptor inhibitor) (Stemgent, Cat. no. 04-0014)	0.5 µM
hLIF (Cat. no. PHC9461)	10 ng/mL
HA-100 (Santa Cruz, Cat. no. sc-203072)	10 µM
bFGF (Cat. no. PHG0264)	100 ng/mL

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

21. What is the recommended medium to use for my reprogrammed cells?

15 days after transfection, the N2B27 Medium with CHALP molecule cocktail and bFGF should be switched to Essential 8™ Medium (Prototype) (Cat. No. A14666SA). The Episomal iPSC Reprogramming Vectors were designed in the laboratories of James Thomson and Cellular Dynamics International for use with Essential 8™ Medium, thus providing an optimal environment for defined, feeder-free reprogramming.

To prepare 500 mL of complete Essential 8™ Medium, thaw Essential 8™ Supplement (50X) at 2–8°C overnight and then aseptically combine the components listed below:

Component	Stock Concentration	Final Concentration	Volume
DMEM/F-12 (HAM) 1:1* (A14625DJ)	—	1X	490 mL
Essential 8™ Supplement (A14626SA)	50X	1X	10 mL

*Note that DMEM/F-12 cannot be used in place of DMEM/F-12 (HAM).

22. Can ROCk inhibitors be used in Essential 8™ Medium?

Yes. However, this isn't necessary and Life Technologies does not routinely use it in their protocols. If the use of ROCk is desired, ROCk is only to be added to the medium at passage. Inhibitors should be removed for routine feeding. Use of inhibitors is assay dependent and not necessary for routine cell culture.

23. What is the role of ROCk inhibitor or Blebbistatin?

The inclusion of either a ROCk inhibitor (HA-100 or Y27632) or blebbistatin improves initial survival and supports a high cloning efficiency which is increased by the addition of transferrin and selenium. If cells are cultured routinely in medium containing ROCk, it may become necessary to include ROCk for routine culture.

24. **Is secondary passaging needed in the derivation of new iPS colonies in the Essential 8™ system?**

    Derivation of new iPS colonies in Essential 8™ Medium and on vitronectin does not require secondary passaging. Once colonies emerge and are sustained, they can be picked directly and transferred to other culture vessels. It is recommended that each picked colony be broken up in smaller pieces and cultured in individual wells (each colony is treated as a clone).

25. **Are the episomal vectors selected out?**

    Episomal vectors can be subsequently removed from cells by culturing them (gradual loss of cellular episomal vectors from proliferating cells).

26. **Can fibroblasts obtained from skin biopsy samples be cultured in Essential 8™ Medium (Prototype) to achieve xeno-free conditions?**

    Yes, fibroblasts from skin biopsy samples can be expanded and cultured in Essential 8™ Medium with the addition of EGF and Thrombin (7).

Expected results with the Episomal iPSC Reprogramming Vectors

27. **How do I confirm that my cells were transfected with the Episomal iPSC Reprogramming Vectors?**

    You can confirm the presence of the Episomal Vectors in your cells by performing RT-PCR for primers that display the presence of episomal vector components oriP and EBNA-1. See the Episomal iPSC Reprogramming Vectors protocol for complete details.

Transgene	Primers	Sequence	Expected Size
oriP	pEP4-SF1-oriP	5'-TTC CAC GAG GGT AGT GAA CC-3'	544 bp
	pEP4-SR1-oriP	5'-TCG GGG GTG TTA GAG ACA AC-3'	
EBNA-1	pEP4-SF2-oriP	5'-ATC GTC AAA GCT GCA CAC AG-3'	666 bp
	pEP4-SR2-oriP	5'-CCC AGG AGT CCC AGT AGT CA-3'	

28. **Should differentiated material be removed prior to passaging?**

    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.

29. **How long does it take to see the iPSC colonies?**

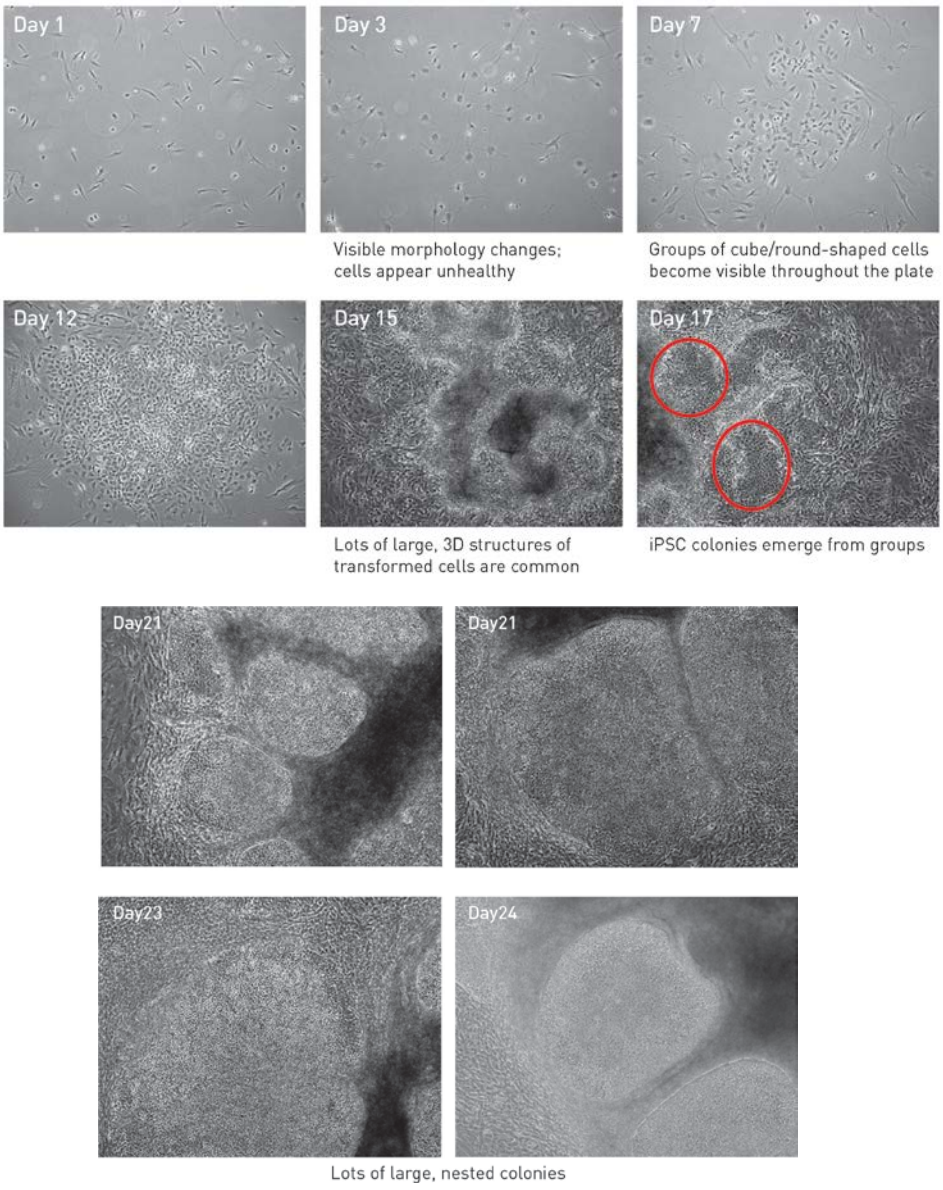
    iPSC colonies should be identifiable by morphology roughly three weeks post transfection.

30. **Why is there a wide range in the number of colonies obtained via this derivation technique?**

    The wide range is due to the variability in the source of fibroblasts [neonatal vs. adult (commercial sources); neonatal vs. adult (patient biopsies)]; age of the donor/patient; and the type and number of reprogramming factors].

31. How will my cells look after I use the Episomal iPSC Reprogramming Vectors?

Time-course images of the generation of iPSCs using the Episomal iPSC Reprogramming Vectors are shown below. The expected morphology of iPSCs is demonstrated specifically by tightly packed colonies with defined borders and a high nucleus-to-cytoplasm ratio.



32. How do I know when to passage the iPSCs?

iPSCs must be monitored and growth medium must be replaced daily in order to maintain a healthy culture. In general, iPSC colonies should be passaged when the cells reach 75–90% confluence or when most of the colonies are larger than 700 µm.

33. Can I cryopreserve the iPSCs?

You can cryopreserve iPSCs just as you would cryopreserve any pluripotent stem cells. Growth medium with 10% DMSO is recommended for freezing.

References:

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