

SNL Feeder Cells

CATALOG NUMBER: MBS169018

STORAGE: Liquid nitrogen

Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80°C until first culture. Store subsequent cultured cells long term in liquid nitrogen.

QUANTITY & CONCENTRATION: 1 mL, 3×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO

Background

Embryonic stem (ES) cells have been derived from the inner cell masses (ICM) of blastocysts in many species. They are capable of unlimited, undifferentiated proliferation on feeder cell layers and remain karyotypically normal and phenotypically stable. In addition, ES cells have the ability to differentiate into a wide variety of cell types *in vitro* and *in vivo*. In mES cell culture, the feeder layer can be replaced by the addition of LIF in the growth medium. However, LIF does not have the same effect on hES cell culture as mES. Therefore, both the derivation and maintenance of hES cells require the use of feeder cells.

SNL 76/7, established by Dr. Allan Bradley (1), is clonally derived from a mouse fibroblast STO cell line transformed with neomycin resistance and murine LIF genes. SNL can be used as a feeder cell for ES cell growth, and it also has been recently used in mouse or human iPS culture (2, 3, 4).

Application

SNL feeder cells are used for the maintenance of ES or iPS cells in the undifferentiated state. The cells must be mitotically inactivated prior to the addition of ES or iPS cells, such as treatment with mitomycin C (2-4 hr, 10 µg/mL).

Quality Control

This cryovial contains at least 3.0×10^6 SNL feeder cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The SNL feeder cells are tested free of microbial contamination.

Medium

1. Culture Medium: D-MEM (high glucose), 10% fetal bovine serum (FBS), 0.1 mM MEM Non-Essential Amino Acids (NEAA), 2 mM L-glutamine, 1% Pen-Strep (optional)
2. Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO

Methods

I. Establishing SNL Feeder Cell Cultures from Frozen Cells

1. Place 10 ml of complete DMEM growth medium in a 50-ml conical tube. Thaw the frozen cryovial of cells by gentle agitation for 1–2 minutes in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
2. Transfer the thawed cell suspension to the conical tube containing 10 mL of growth medium.
3. Collect the cells by centrifugation at 1000 rpm for 5 minutes at room temperature. Remove the growth medium by aspiration.
4. Resuspend the cells in the conical tube in 15 mL of fresh growth medium by gently pipetting up and down.
5. Transfer the 15 mL cell suspension to a T-75 tissue culture flask. Place the cells in a 37°C incubator at 5% CO₂.
6. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

II. Freezing SNL Feeder Cells

1. Trypsinize cells and resuspend cell pellet in cold Freeze Medium at twice the desired final cell concentration.
2. Aliquot 1 mL of cells into sterile cryovials and place cryovials immediately into freezing container. Store overnight at -80°C.
3. Transfer frozen vials to -135°C freezer or liquid nitrogen.

III. Mitomycin C Treatment and Preparation of Feeder

1. Culture cells to 90% confluence. Wash it once with sterile PBS.
2. Add 10 µg/mL Mitomycin C (Sigma), incubate for 2 hrs.
3. Wash 3 times with sterile PBS to remove Mitomycin.
4. After dissociation by Trypsin, the Mitomycin-treated SNLs can be freeze-dried and stored in liquid nitrogen, or used as feeder by plating them at 75 000 cells/cm² in gelatin-coated tissue culture dishes for one day.

References

1. McMahon, A.P. and Bradley, A. (1990) *Cell* **62**:1073–1085.
2. Okita, K; Ichisaka, T; Yamanaka, S. (2007) *Nature* **448**:313–317.
3. Takahashi K, Okita K, Nakagawa M, Yamanaka S. (2007) *Nat Protoc.* **2**:3081-9.
4. Takahashi K, Narita M, Yokura M, Ichisaka T, Yamanaka S. (2009) *PLoS One* **4**(12):e8067.

Recent Product Citations

1. Hayashi, Y. et al. (2018). Automated adherent cell elimination by a high-speed laser mediated by a light-responsive polymer. *Commun Biol.* **1**:218. doi: 10.1038/s42003-018-0222-4.
2. Horikawa I, et al. (2017). Δ133p53 represses p53-inducible senescence genes and enhances the generation of human induced pluripotent stem cells. *Cell Death Differ.* **24**(6):1017-1028. doi: 10.1038/cdd.2017.48.
3. Mitzelfelt, K. A. et al. (2016). Human 343delT HSPB5 chaperone associated with early-onset skeletal myopathy causes defects in protein solubility. *J Biol Chem.* doi:10.1074/jbc.M116.730481.

4. Shiba, Y. et al. (2016). Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. *Nature* 10.1038/nature19815.
5. Saito, H. et al. (2016). Adoptive transfer of CD8⁺ T cells generated from induced pluripotent stem cells triggers regressions of large tumors along with immunological memory. *Cancer Res.* doi:10.1158/0008-5472.CAN-15-1742.
6. Saito, H. et al. (2015). Reprogramming of melanoma tumor-infiltrating lymphocytes to induced pluripotent stem cells. *Stem Cells Int.* 292385.
7. Arai, Y. et al. (2015). Spectral fingerprinting of individual cells visualized by cavity-reflection-enhanced light-absorption microscopy. *PLoS One.* **10**:e0125733.
8. Wu, D. T. & Roth, M. J. (2014). MLV based viral-like-particles for delivery of toxic proteins and nuclear transcription factors. *Biomaterials.* **35**:8416-8426.
9. Takenaka-Ninagawa, N. et al. (2014). Generation of rat-induced pluripotent stem cells from a new model of metabolic syndrome. *PLoS One.* **9**:e104462.
10. Piovan, C. et al. (2014). Generation of mouse lines conditionally over-expressing microRNA using the Rosa26-Lox-Stop-Lox system. *Methods Mol Biol.* **1194**:203-224.
11. Kuroda, T. et al. (2014). In vitro detection of residual undifferentiated cells in retinal pigment epithelial cells derived from human induced pluripotent stem cells. *Methods Mol Biol.* **1210**:183-192.
12. Graversen, V. K. & Chavala, S. H. (2014). Induced pluripotent stem cells: generation, characterization, and differentiation-methods and protocols. *Methods Mol Biol.* 10.1007/7651_2014_148
13. Ni, A. et al. (2014). Sphere formation permits Oct4 reprogramming of ciliary body epithelial cells into induced pluripotent stem cells. *Stem Cells Dev.* **23**:3065-3071.
14. Yi, L. et al. (2012). Multiple roles of p53-related pathways in somatic cell reprogramming and stem cell differentiation. *Cancer Res.* **72**: 5635-5645.