Applied StemCell, Inc.
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Datasheet

SNL 76/7 Feeder Cells, P12, Untreated

Product Information

Specifications

Catalog Number	Cells per Vial	Treatment	Number of Vials
ASF-1305	2 x 10^6	Untreated	1

Description

Mouse embryonic stem cells (ESCs) and induce pluripotent stem cells (iPSCs) generally require culture on feeder cells as well as medium containing Leukemia Inhibitory Factor (LIF) to maintain pluripotency and the capacity to self-renew. The SNL 76/7 cell line, derived from a mouse fibroblast STO cell line transformed with neomycin resistance and murine LIF genes, can be used as feeder cells for mouse and human ESC and iPSC culture. The SNL cells are sensitive to HAT selection (hypoxanthine, aminoprotein and thymidine) and negative for HPRT (hypoxanthine guanine phosphoribosyl transferase). Before use, SNL 76/7 cells must be mitotically inactivated by γ-irradiation or mitomycin-C treatment.

Passage

P12

Treatment

Untreated

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Biosafety Level

BSL-1

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

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Media and Material

Medium

Component	Concentration	Vendor
ESC-Sure™ DMEM		Applied StemCell, #ASM-5001
ESC-Sure™ FBS	10%	Applied StemCell, #ASM-5007
Nonessential amino acids	0.1 mM	Life Technologies, #11140-050
Sodium Pyruvate	1 mM	Life Technologies, #11360-070
L-Glutamine	2 mM	Life Technologies, #25030-164

Suggested Neomycin (G418) concentration: 200 µg/mL

Protocol

1. Remove a vial of frozen cells from liquid nitrogen and place it onto dry ice for 5' before thawing it at 37 ºC water bath. As soon as the majority of the content of the vial thawed, transfer it to a conical tube containing 10x volume of pre-warmed medium.

2. Spin at 1000 rpm for 5 min at 4º C, discard medium, resuspend the cells in growth medium and plate them in a culture vessel at a density of ~25,000 cells/cm^2.

3. After 2-3 days (cells should have grown to a density of ~150,000 cells/cm^2), trypsinize the cells and subculture at 1:3 ratio.

4. For use as feeder cells, plate mitotically inactivated cells (see below) at an appropriate density in a gelatin-coated tissue-culture dish (generally 75,000 cells/cm^2. Optimal density is to be determined by the user for specific applications.

- **Mitotic inactivation by γ-irradiation**
  When cells reach confluency, trypsinize the cells, spin down, resuspend cells in chilled growth medium, and γ-irradiate the cell suspension at 3000 rad.

- **Mitotic inactivation by Mitomycin-C treatment**
  When cells are confluent, treat the cells with 10 µg/ml mitomycin C for 2 hours, then trypsinize the cells, spin down and resuspend in growth medium and plate for use (cells can also be frozen down in freezing medium).

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.