JK1 Feeder Cells

CATALOG NUMBER: CBA-315 **STORAGE:** Liquid Nitrogen

QUANTITY AND CONCENTRATION: 1 mL, >1 x 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*. Human ESCs are normally maintained on MEFs in conjunction with media containing a serum replacement. Human ESCs can also be cultured feeder-free on Matrigel®, laminin, or fibronectin in MEF-conditioned medium. Unfortunately, MEFs can be only passaged approximately five times before undergoing senescence. This requires the fresh derivation of MEFs in large quantities from mice and the preparation of a frozen stock which can be thawed, growth inactivated, and used as feeder cells when needed. This process is not only cumbersome but also requires a significant devotion of time and reagents, most often resulting in the generation of a heterogeneous population of stromal cells that varies in its capacity to support stem cell expansion.

JK1 is an murine immortalized SMA^+ $CD34^+$ testicular stromal cell line. It supports long-term proliferation of numerous types of stem cells including pluripotent ESCs, germ line-derived stem cells such as spermatogonial progenitor cells (SPCs) and multipotent adult spermatogonial-derived stem cells (MASCs), and primordial germ cell (PGC)-derived embryonic germ cells (EGCs). The JK1 feeder line has maintained its capacity for promoting stem cell self-renewal even after serial passaging during the course of more than one year (Ref. 1).

Application

JK1 feeder cells are used for the maintenance of numerous types of stem cells in their undifferentiated state. The cells must be mitotically inactivated prior to the addition of ES cells, such as treatment with mitomycin C (2-4 hr, 10 µg/mL).

Quality Control

This cryovial contains at least 1.0 × 10^6 JK1 feeder cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The MEF 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

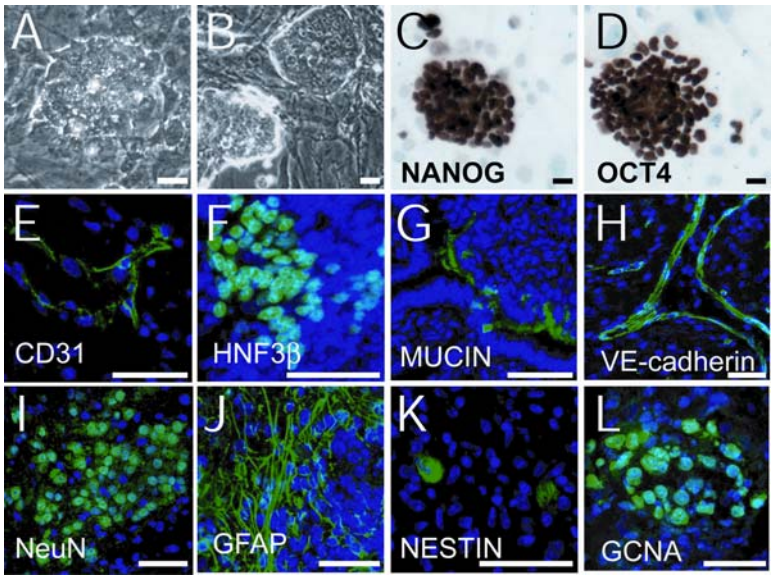
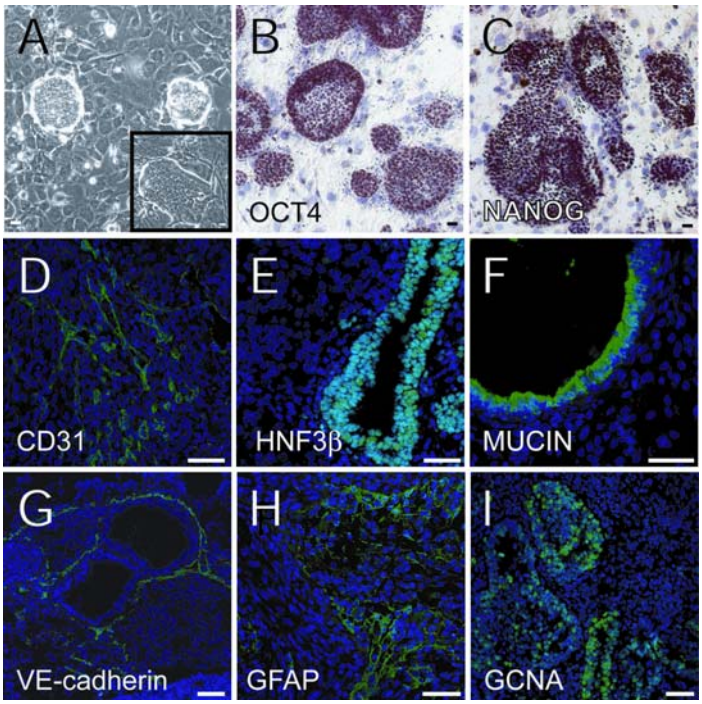


Figure 1. JK1 cells support the maintenance of murine embryonic stem cells. **(A–B):** Phase-contrast images (original magnification, x200) of murine ESC colonies on **(A)** JK1 cells and **(B)** mouse embryonic fibroblasts (MEFs) showed a similar, characteristic ESC morphology: tight, round colonies of cells with a high nucleus to cytoplasm ratio. **(C–D):** Immunohistochemistry (brown staining) showed expression of **(C)** NANOG, and **(D)** OCT4 in ESCs was maintained after culturing on JK1 cells for more than 1 month. **(E–L)**: Immunofluorescence demonstrated that mESC-derived teratomas expressed markers of all three germ layers: **(E)** CD31, **(F)** HNF3ß, **(G)** mucin, **(H)** VE-cadherin, **(I)** NeuN, **(J)** GFAP, **(K)** Nestin, and **(L)** GCNA. Antibody staining is green. Nuclear counterstain is blue.

Figure 2. Multipotent adult spermatogonial-derived stem cells (MASCs) cultured on JK1 cells maintain multipotency. **(A):** Phase-contrast images of MASC colonies on JK1 cells (**A** inset) and mouse embryonic fibroblasts (original magnification, x100). **(B–C):** Immunohistochemistry revealed **(B)** OCT4 and **(C)** NANOG expression was preserved in MASCs passaged on JK1 cells for more than 2 weeks. **(D–I):** MASC-derived teratomas expressed markers for all three germ layers by immunofluorescence. **(D)** CD31, **(E)** HNF3β, **(F)** mucin, **(G)** VE-cadherin, **(H)** GFAP, and **(I)** GCNA. Antibody staining is green. Nuclear counterstain is blue.



Methods

I. Establishing JK1 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 within 1–2 minutes by gentle agitation 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 of 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 split at 1:4 – 1:6 ratio when the culture reaches 95% confluence.

II. Freezing JK1 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 -80ºC.
3. Transfer frozen vials to -135ºC freezer or liquid nitrogen.

III. Mitomycin C Treatment and Preparation of JK1 Feeder Cells

1. Culture cells to 90% confluence. Wash once with sterile PBS.
2. Add 10 µg/mL Mitomycin C (Sigma) and incubate for 2 hrs.
3. Wash 3 times with sterile PBS to remove Mitomycin.
4. After dissociation by Trypsin, the Mitomycin-treated JK1 cells can be frozen and stored in liquid nitrogen, or used as feeders by plating them at 75000 cells/cm^2 in gelatin-coated tissue culture dishes for one day.

References

1. Kim, J. et al. (2008). CD34+ Testicular Stromal Cells Support Long-Term Expansion of Embryonic and Adult Stem and Progenitor Cells. *Stem Cells* **26**: 2516-2522.

Warranty

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

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