EmbryoMax® JK1 Murine Testicular Stromal Feeder Cell Line

Immortalized Cell Line

Cat. # SCC169

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Pack size: ≥1x10^6 viable cells/vial

Store in liquid nitrogen

Data Sheet

Background:

JK1 is an immortalized CD34+SMA+ cell line derived from spontaneuos transformation of C57/BL6 mouse testicular stromal cells. JK1 cells is able to support the long-term culture of a variety of primitive cells including multi-potent adult spermatogonial-derived stem cells, pluripotent murine embyronic stem cells and embyronic germ cells derived from primordial germ cells[1].

Adult spermatogonial stem and progenitor cells (SPCs) cultured on JK1 feeder cells express key features of germ line stem cells including expression of PLZF, DAZL, and GCNA[1]. JK1 feeder cells has maintained its capacity for promoting stem cell self-renewal even after serial passaging for over one year[1].

Storage and Handling

EmbryoMax® JK1 Murine Testicular Stromal Feeder Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for inter-species contamination from rat, chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells are negative for mycoplasma contamination.

Representative Data

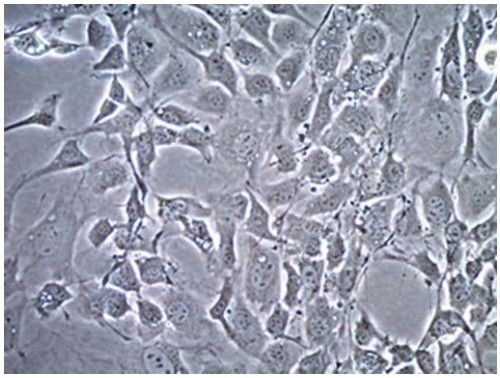

Figure 1. JK1 cells one day after thaw.

References

1. Kim J, Seandel M, Falciatori I, Wen D, Rafii S. (2008) CD34+ testicular stromal cells support long-term expansion of embryonic and adult stem and progenitor cells. *Stem Cells* 26(10): 2516-2522.

Rev.1.0/2018-03-09/SCC169/VC

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in DMEM Complete Medium (Cat. No. SLM-241-B) **or** in DMEM-High Glucose (Sigma Cat. No. 6546), 10% FBS (Cat. No. ES-009-B) and 2 mM L-Glutamine (Cat. No. TMS-002-C) and 1X Penicillin-Streptomycin Solution (Cat. No. TMS-AB2-C) (optional).
2. Remove the vial of frozen JK1 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of JK1 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 45 – 50 mL of JK1 Expansion Medium.
10. Transfer the cell mixture to a **T225** tissue culture flask.

    Note: *JK1 cells proliferate extremely rapidly. Do not thaw into a T75 flask.*
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. Carefully remove the medium from the T225 tissue culture flask containing the confluent layer of JK1 cells.
2. Rinse the T225 flask twice with 20 mL 1X PBS. Aspirate after each rinse.
3. Apply 10 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 12 mL of JK1 Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 50 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of JK1 Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density (typical split ratio is 1:6 – 1:9). Cells proliferate extremely rapidly.

Cryopreservation of Cells

JK1 Murine Testicular Stromal Feeder Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

Mitomycin C Treatment of JK1 cells

1. Remove the culture medium from the confluent JK1 monolayer.
2. Wash cells once with 1X PBS.
3. Add Accutase or Trypsin-EDTA and incubate at 37°C for 5-7 minutes.
4. Tap firmly on the sides of the flask to dislodge the cells. Add an equal volume of serum-containing medium to inactivate the trypsin-EDTA.
5. Triturate the detached layer and collect the cells.
6. Centrifuge the cells at 300g for 5 minutes. Resuspend the cell pellet in JK1 Expansion Medium.
7. Plate ~250 cells per mm^2 tissue culture plate (pre-coated with gelatin). Incubate culture at 37°C for 15-24 hours.

   Note: Optimal plating density of JK1 feeder cells may vary with different stem cells and must be determined empirically.
8. After 15-24 hours, remove the culture medium from the wells. Add 1 mL of 10 μg/mL Mitomycin-C (Sigma Cat. No. M-4287-2MG) diluted in the culture medium to each well. Incubate at 37°C for 2.5 hours (not more than 3.5 hours).

   Note: Mitomycin C is toxic. Please collect waste for proper disposal.
9. Remove the Mitomycin-C solution from the cells. Wash 3 times with plain DMEM (i.e. without serum or other additives).
10. Return treated cells to JK1 Expansion Medium (i.e. containing 10% FBS) at 37°C. Mitomycin C-treated JK1 cells in JK1 Expansion Medium can be used as feeders within 3 days after treatment.
11. Remove culture medium from JK1 wells. Plate stem cells on top of the monolayer in stem cell medium. Incubate at 37°C.

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