CF6-Neo Mouse Embryonic Fibroblasts, MitC-Treated

Catalog Number A34964

Pub. No. MAN0016997 **Rev.** 1.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Mitomycin C (MitC)-treated CF6-Neo Mouse Embryonic Fibroblasts (MEFs) are neomycin-resistant feeder cells that support healthy undifferentiated human and mouse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) in culture. These MEF cells are isolated from F1 (CF-1 x C57BL/6J-Tg (pPGKneobpA)3Ems/J) mice, expanded for up to three passages, mitotically inactivated by MitC treatment, and rigorously tested to ensure safety, performance, and resistance to geneticin (G418). These ready-to-use MEFs help save time. In addition, they also enable researchers to confidently perform geneticin selection while using feeder-dependent ESCs and iPSCs.

Contents and storage

Contents	Catalog No.	Amount	Storage
CF6-Neo Mouse Embryonic Fibroblasts, MitC-Treated	A34964	1 mL (≥4 × 10^6 viable cells/mL)	Liquid nitrogen (vapor phase)

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
DMEM, high glucose, GlutaMAX™ Supplement, pyruvate	10569010
DPBS, no calcium, no magnesium	14190144
Fetal Bovine Serum, embryonic stem cell-qualified	16141061
Attachment Factor Protein (1X)	S006100

Culture conditions

Media: DMEM, high glucose, GlutaMAX™ Supplement, pyruvate, supplemented with 10% Fetal Bovine Serum, embryonic stem cell-qualified.

Culture type: Adherent

Recommended substrate (optional): Attachment Factor, which is a sterile 1X solution containing 0.1 % gelatin.

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2 in air.

Procedural guidelines

Follow the guidelines below to use inactivated mouse embryonic fibroblasts (MEFs) as feeder layers to culture mouse and human ESCs and iPSCs.

- All solutions and equipment that come in contact with the cells must be sterile. Always use proper aseptic technique and work in a laminar flow hood.
- MEFs should be plated ~24 hours in advance.
- After thawing, transfer MEFs into pre-warmed medium.
- Plate MEFs on culture vessels coated with Attachment Factor Protein (1X) solution.
- For best results, use MEF dishes or plates the day after seeding and culture with ESCs or iPSCs for up to 4 more days.

Before you begin

Before starting experiments, make sure to have some frozen ESC or iPSC stocks on hand.

Coat culture vessels with Attachment Factor

1. Cover the whole surface of each culture vessel with Attachment Factor (AF) solution.

Vessel size	AF coating volume
96-well plate	0.1 mL
24-well plate	0.3 mL
12-well plate	0.5 mL
6-well plate	1 mL
60-mm dish	3 mL
100-mm dish	9 mL
25-cm^2 flask	3 mL
75-cm^2 flask	9 mL

2. Incubate the vessels for 30 minutes at 37°C or for 2 hours at room temperature.

3. Using sterile technique in a laminar flow culture hood, completely remove the AF solution from the culture vessel by aspiration.

   Note: It is not necessary to wash the culture surface before adding cells or medium.

4. Use the coated vessels immediately or store them at room temperature for up to 24 hours.

Prepare MEF Medium

Prepare 500 mL of MEF Medium by mixing the following components (pre-warmed in a 37°C, 5% CO_2 incubator):

Component	Amount
DMEM, high glucose, GlutaMAX™ Supplement, pyruvate	450 mL
Fetal Bovine Serum, embryonic stem cell-qualified	50 mL

Thaw MEFs

1. Remove the cryovial containing inactivated MEFs from the liquid nitrogen storage tank.

2. Briefly roll the vial between hands to remove frost, and swirl it gently in a 37°C water bath.

3. When only a small ice crystal remains in the vial, remove it from water bath. Spray the outside of the vial with 70% ethanol before placing it in the cell culture hood.

4. Pipet the thawed cells gently into a 50-mL conical tube.

5. Add 10 mL of pre-warmed MEF Medium dropwise to the cells while gently swirling the conical tube. Gently mix by pipetting up and down.

   Note: Adding the medium slowly helps the cells to avoid osmotic shock.

6. Transfer entire cell suspension to a 15-mL conical tube and centrifuge at 200 × g for 5 minutes.

7. Aspirate the supernatant and resuspend the cell pellet in an appropriate volume of pre-warmed MEF Medium.

8. Use an appropriate volume of the cell suspension to determine the viable cell number using your method of choice.

Plate MEFs

1. Aspirate the gelatin solution from the AF-coated culture vessels, as applicable.

2. Add the appropriate amount of MEF Medium into each culture vessel.

Vessel size	MEF Medium volume
96-well plate	0.1 mL
24-well plate	0.5 mL
12-well plate	1 mL
6-well plate	2 mL
60-mm dish	5 mL
100-mm dish	10 mL
25-cm^2 flask	5 mL
75-cm^2 flask	15 mL

3. Add the appropriate amount of MEF suspension into each culture vessel.

Vessel size	Number of MEFs
96-well plate	1.0×10^4 cells/well
24-well plate	6.0×10^4 cells/well
12-well plate	1.5×10^5 cells/well
6-well plate	3.0×10^5 cells/well
60-mm dish	6.0×10^5 cells
100-mm dish	1.8×10^6 cells
25-cm^2 flask	7.5×10^5 cells
75-cm^2 flask	2.3×10^6 cells

Note: The appropriate cell density should be optimized for the specific application. We recommend 3.0×10^4 MEFs/cm^2 as a good starting point, but the typical range is 2.0×10^4 to 5.5×10^4 MEFs/cm^2.

4. Move the culture vessels in several quick back-and-forth and side-to-side motions to disperse the cells across the surface of the vessels.

5. Incubate the cells in a 37°C incubator with a humidified atmosphere of 5% CO_2.

6. Use the MEF culture vessels the day after plating.

Expected results

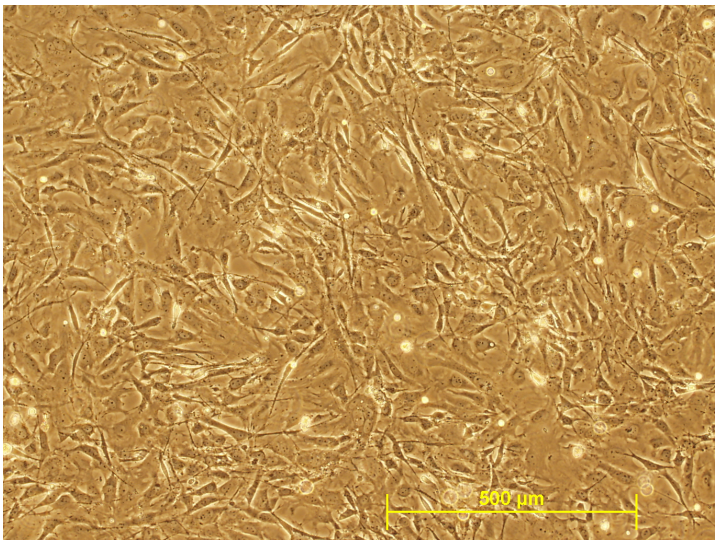

Figure 1 Mitomycin C-treated CF6-Neo Mouse Embryonic Fibroblasts

Mitomycin C-treated CF6-Neo MEFs were cultured in DMEM supplemented with FBS. Image was taken with a 10x objective.

Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer	REF	Catalog number	LOT	Batch code
	Use by		Temperature limitation		

Limited product warranty

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Manufacturer's address: Made in USA by MTI-GlobalStem | 7335 Executive Way | Frederick, MD 21704 | USA

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