Mouse (ICR) inactivated embryonic fibroblasts

Catalog Number A24903

Pub. No. MAN0010648 Rev. B.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**.

Description

Mouse (ICR) Inactivated Embryonic Fibroblasts are used as feeder layers for culturing embryonic stem cells (ESCs), including mouse and human ESCs, in their undifferentiated state. The fibroblasts were isolated from ICR mouse embryos under sterile conditions, expanded for up to two passages, and then mitotically inactivated by γ-irradiation. The growth-arrested feeder layer supports the ESC culture by providing nutrients, growth factors, and matrix components, and it enables ESCs to survive and proliferate more readily in culture.

Product	Catalog No.	Amount	Storage
Mouse (ICR) Inactivated Embryonic Fibroblasts	A24903	1 mL (≥2 × 10^6 viable cells/mL)	Liquid nitrogen, vapor-phase

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.

Important information

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

- **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.
- Make sure to prepare MEF feeder layer at least one day before culturing ESCs.
- After thawing, transfer MEFs into pre-warmed medium.
- Plate MEFs on culture vessels coated with 1× Attachment Factor (AF) solution.
- Use MEF dishes or plates within 3–4 days of preparation.
- Before starting experiments, ensure that ESCs have been established (at least 1 passage), and also have some frozen ESC stocks on hand.

Culture conditions

Media: Dulbecco's Modified Eagle medium (DMEM) containing 4.5 g/L glucose (Cat. no. 10569), and supplemented with 10% FBS, ES-cell qualified (Cat. no. 16141).

Culture Type: Adherent

Recommended Substrate: Attachment Factor (Cat. no. S-006-100), which is a sterile 1× solution containing 0.1 % gelatin.

Temperature Range: 36℃ to 38℃

Incubator Atmosphere: Humidified atmosphere of 5% CO_2.

Coat culture vessels with attachment factor

1. Cover the whole surface of each culture vessel with Attachment Factor (AF) solution and incubate the vessels for 30 minutes at 37℃ or for 2 hours at room temperature. Refer to the recommended coating volumes in the table, next page.

2. 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.

3. You can 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℃, 5% CO_2 incubator):

D-MEM™	450 mL
FBS	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℃ 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

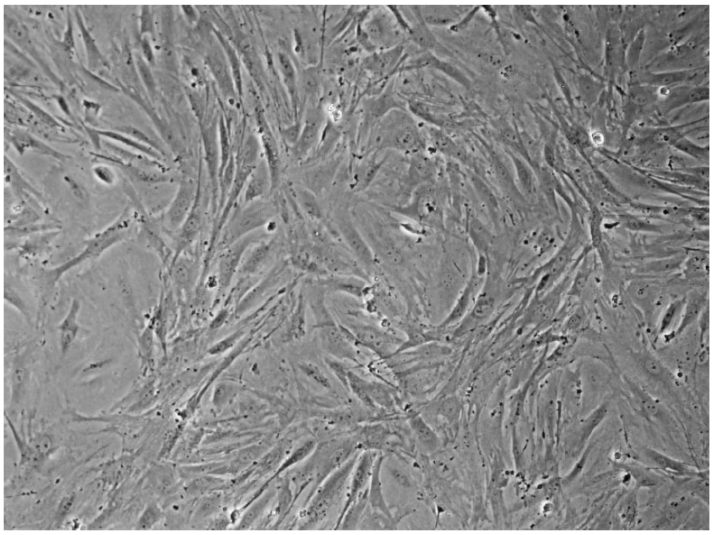
1. Aspirate the gelatin solution from the AF-coated culture vessels.
2. Add the appropriate amount of MEF medium into each culture vessel (refer to the table, next page).
3. Into each of these culture vessels, add the appropriate amount of MEF suspension (refer to the table, next page).

   Note: The appropriate cell density should be optimized for the specific application. We recommend 3.0 × 104 MEFs/cm^2 as a good starting point.
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℃ incubator with a humidified atmosphere of 5% CO_2.
6. Use the MEF culture vessels within 3–4 days after plating.

Vessel Size	AF Coating Volume	Volume of Media	Number of MEFs
96-well plate	0.1 mL	0.1 mL	1.0 × 104/well
24-well plate	0.3 mL	0.5 mL	6.0 × 104/well
12-well plate	0.5 mL	1 mL	1.1 × 105/well
6-well plate	1 mL	2 mL	2.9 × 105/well
60-mm dish	3 mL	5 mL	5.9 × 105
100-mm dish	9 mL	10 mL	1.8 × 106
25-cm^2 flask	3 mL	5 mL	7.5 × 105
75-cm^2 flask	9 mL	15 mL	2.3 × 106

Expected results

The bright field image below shows Mouse (ICR) Inactivated Embryonic Fibroblasts plated at the recommended density on culture dishes coated with 1× AF solution. The image was taken with a 10× objective.



Related products

Product	Cat. no.
Dulbecco's Modified Eagle Medium (D-MEM™), high glucose	A10569
Dulbecco's PBS (DPBS) without Calcium and Magnesium	14190
Fetal Bovine Serum (FBS), ES-Cell Qualified	A16141
Attachment Factor	S-006-100

References

Evans, M., Kaufman, M. (1981) Establishment in culture of pluripotent cells from mouse embryos. Nature *292*, 154–156.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., Jones, J.M. (1998) Embryonic stem cell lines derived from human blastocysts. Science *282*, 1145–1147.

Williams, R.L., Hilton, D.J., Pease, S., Willson, T.A., Stewart, C.L., Gearing, D.P., Wagner, E.F., Metcalf, D., Nicola, N.A., Gough, N.M. (1988) Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature *336*, 684–687.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.