OriCell Strain C57BL/6 Mouse Embryonic Stem Cells/GFP

Catalog No. MUBES-01101

Instructions for Use

Materials Required (not supplied)

1. Gelatin Solution (Cat. No. GLT-11301-100)
2. Mouse Embryonic Fibroblast Growth Medium (Cat. No. MUXEF-90011)
3. Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium (Cat. No. MUBES-90011)
4. Phosphate-Buffered Saline (1×PBS) (Cat. No. PBS-10001-500)
5. Trypsin-EDTA (Cat. No. TEDTA-10001-100）
6. Strain ICR Mouse Embryonic Fibroblasts (Cat. No. MUIEF-01002)

Gelatin Coating of Tissue Culture Vessels

1. Add sufficient Gelatin Solution into the culture vessel to completely cover its base.
2. Swirl until Gelatin Solution coats entire base of vessel. Let sit for at least 30 minutes at room temperature.
3. Aspirate off all of the Gelatin Solution and allow the remainder to evaporate by leaving the vessel sitting open in the hood for no more than 30 minutes.
4. Put lid back once the surface is dry.

Note: Gelatinized dishes or flasks can be stored at 4°C for at most 2 weeks, provided they remain sterile.

Thawing of γ-ray Irradiated Strain ICR Mouse Embryonic Fibroblasts (feeder cells/MEF)

1. Prepare 37°C water bath and pre-warm the Mouse Embryonic Fibroblast Growth Medium to 37°C.
2. Add 9 mL of Mouse Embryonic Fibroblast Growth Medium to a 15 mL conical tube.
3. Remove the cryovial of Strain ICR Mouse Embryonic Fibroblasts from liquid nitrogen. Quickly thaw the vial in 37°C water bath until the last crystal piece disappears, and finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

Note: Thawing the cells for longer than 3 minutes results in less than optimal results.

4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol.
5. In a laminar flow hood, use pipette to transfer the cells to the conical tube containing Mouse Embryonic Fibroblast Growth Medium. Be careful not to introduce any bubbles during the transfer process.
6. Rinse the vial with 1 mL of medium to reduce the loss of cell and then transfer the cell suspension to the conical tube.
7. Gently mix the cell suspension by slowly pipeting up and down. Be careful not to introduce any bubbles.
8. Centrifuge the cell suspension at 250 g for 5 minutes.
9. Carefully aspirate as much of the supernatant as possible and add 3 mL of fresh Mouse Embryonic Fibroblast Growth Medium (pre-warmed to 37°C).
10. Gently re-suspend the cells in Mouse Embryonic Fibroblast Growth Medium.
11. Plate the cell suspension into wells of one 6-well plates pre-coated with Gelatin Solution (or other appropriate flasks) and add sufficient Mouse Embryonic Fibroblast Growth Medium. Gently rock the culture plate to evenly distribute the cells.
12. Incubate at 37°C in a 5% CO_2 humidified incubator.
13. The next day, change the medium with fresh Mouse Embryonic Fibroblast Growth Medium (pre-warmed to 37°C). If the next day thawing of the Strain C57BL/6 Mouse Embryonic Stem Cells/GFP is performed, the medium can be change directly to Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium.

Note:

1. **Thawing the feeder cells should be performed at least one day before thawing the Strain C57BL/6 Mouse Embryonic Stem Cells/GFP.**
2. **The feeder cells should be used as soon as possible once thawed.**

Thawing of Strain C57BL/6 Mouse Embryonic Stem Cells/GFP

1. Prepare 37°C water bath and pre-warm the Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium, 1×PBS to 37°C.
2. Add 9 mL of Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium to a 15 mL conical tube.
3. Remove the cryovial of Strain C57BL/6 Mouse Embryonic Stem Cells/GFP from liquid nitrogen. Quickly thaw the vial in 37°C water bath until the last crystal piece disappears, and finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell

viability is dependent on the rapid and complete thawing of frozen cells.

Note: Thawing the cells for longer than 3 minutes results in less than optimal results.

4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol.
5. In a laminar flow hood, use pipette to transfer the cells to the conical tube containing Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium. Be careful not to introduce any bubbles during the transfer process.
6. Rinse the vial with 1 mL of medium to reduce the loss of cell and then transfer the cell suspension to the conical tube.
7. Gently mix the cell suspension by slowly pipeting up and down. Be careful not to introduce any bubbles.
8. Centrifuge the cell suspensions at 250 g for 5 minutes.
9. Carefully aspirate as much of the supernatant as possible and add 3 mL of fresh Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium (pre-warmed to 37°C).
10. Gently re-suspend the cells in Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium.
11. Plate the cells into two T25 flasks and add sufficient Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium. Gently rock the culture flask to evenly distribute the cells.
12. Incubate at 37°C in a 5% CO_2 humidified incubator.
13. The next day, change the medium with fresh Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium (pre-warmed to 37°C).

Changing Medium

1. Warm an appropriate amount of Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium to 37°C in a sterile container. Remove the spent medium and replace it with the warmed, fresh medium and return the flask to the incubator.
2. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer only the required volume to a sterile secondary container.

Subculturing

1. Pre-warm Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium, 1×PBS, Trypsin-EDTA solution to 37°C.
2. Aspirate the spent medium from the Strain ICR Mouse Embryonic Fibroblasts (MEF).
3. Rinse MEF with 1×PBS (3 mL for one well of six-well plate).
4. Aspirate the 1×PBS from the flask and discard.

5. Repeat step 3-4 one or two times.
6. Add the pre-warmed Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium. Return the MEF to the 5% CO_2 humidified incubator.

Note: Be careful not to disturb the monolayer of MEF during step 2-6.

7. Carefully aspirate off spent medium from Strain C57BL/6 Mouse Embryonic Stem Cells/GFP.
8. Rinse the cells with 1×PBS (3 ml for one well of six-well plate).
9. Aspirate the 1×PBS from the flask and discard.
10. Repeat the step 8-9 two or three times.
11. Add Trypsin-EDTA solution(200 μL for one well of six-well plate) and incubate for 1-2 minutes, until the Strain C57BL/6 Mouse Embryonic Stem Cells/GFP are dissociated. At this point, gently tap the side of the flask to release the majority of cells from the culture surface.
12. Add Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium (3 mL for one well of six-well plate) and gently pipette up and down until colonies become dissociated to single cells.

Note: Be careful not to introduce any bubbles.

13. Transfer the dissociated cells into a 15 mL conical tube.
14. Centrifuge the tube at 250 g for 5 minutes to pellet the cells.
15. Carefully aspirate as much of the supernatant as possible.
16. Add 2 ml of Strain C57BL/6 Mouse Embryonic Stem Cell Growth Medium to the conical tube and re-suspend the cells thoroughly but gently.
17. Plate the cells into flasks containing the MEF. Split ratios for Strain C57BL/6 Mouse Embryonic Stem Cells/GFP can vary from 1:3 to 1:10.
18. Add sufficient medium.
19. Incubate the cells at 37°C in a 5% CO_2 humidified incubator until it is time to split again. We typically split Strain C57BL/6 Mouse Embryonic Stem Cells/GFP every other day.

Note:

1. **Embryonic stem cells should be plated at a density that provides an even distribution of colonies over the surface, but does not result in contact between the colonies. Differentiation can occur if the colonies are plated too densely or too sparsely.**
2. **Strain C57BL/6 Mouse Embryonic Stem Cells/GFP should not be over-subcultured, minimize the number of passages and the length of time the cells are kept in culture. This will ensure enhanced and reproducible experimental results.**

Hints

Time to Split Strain C57BL/6 Mouse Embryonic Stem Cells/GFP

Passage the cells before the colonies become too large and dense. When plated at the optimum density, Strain C57BL/6 Mouse Embryonic Stem Cells/GFP should be passaged every 48 hours.