OriCell™ Human Embryonic Stem Cells

Catalog No. HUXES-01001

Instrutions 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. Human Embryonic Stem Cell Growth Medium (Cat. No. HUXES-90011)
4. Phosphate-Buffered Saline (1×PBS) (Cat. No. PBS-10001-500)
5. Collagenase IV (Cat. No. COL-001-100)
6. Strain Kunming Mouse Embryonic Fibroblasts (Cat. No. MUKEF-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 Kunming 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 Kunming 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 two 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 Human Embryonic Stem Cells is performed, the medium can be change directly to Human Embryonic Stem Cell Growth Medium.

Note:

1. **Thawing the feeder cells should be performed at least one day before thawing Human Embryonic Stem Cells.**
2. **The feeder cells should be used as soon as possible once thawed.**

Thawing of Human Embryonic Stem Cells

1. Prepare 37°C water bath and pre-warm the Human Embryonic Stem Cell Growth Medium, 1×PBS to 37°C.
2. Add 9 mL of Human Embryonic Stem Cell Growth Medium to a 15 mL conical tube.
3. Remove the cryovial of Human Embryonic Stem Cells 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 Human 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 Human Embryonic Stem Cell Growth Medium (pre-warmed to 37°C).
10. Gently re-suspend the cells in Human Embryonic Stem Cell Growth Medium.
11. Plate the cells into one well of six-well plate and add sufficient Human 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. Refeed cells the following day and daily thereafter.

Note： Recovery efficiency of Human Embryonic Stem Cells is about 0.1 to 1 percent. You may not see visible colonies immediately and the colonies usually begin to appear in 4 to 5 days.

Changing Medium

1. Warm an appropriate amount of Human 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 Human Embryonic Stem Cell Growth Medium, 1×PBS, Collagenase IV solution to 37°C.
2. Aspirate the spent medium from the Strain Kunming 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 Human 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 spent medium from Human Embryonic Stem Cells.
8. Add 1 mL Collagenase IV solution to each well of the six-well plate.
9. Incubate for at least 5 minutes.
10. Inspect the plate under a microscope and ensure the perimeter of the colony to appear folded back.
11. Using a glass pipet, scrape cells off the surface of the plate.
12. While scraping the cells off the plate, slowly pipet the Collagenase IV solution up and down to wash the cells off the surface.
13. Transfer the dissociated cells into a 15 mL conical tube.
14. Gently pipet cells up and down a few times in the 15 mL conical tube to further break-up cell colonies. Be careful to not break up the colonies into a single cell suspension.

15. Centrifuge the tube at 250 g for 5 minutes to pellet the cells.
16. Carefully aspirate as much of the supernatant as possible. Re-suspend pellet with 2-3mL Human Embryonic Stem Cell Growth Medium.
17. Plate the cells into wells of 6-well tissue culture plate containing MEF.

Note: Human Embryonic Stem Cells can be split at 1:6 or other appropriate ratio.

18. Add sufficient medium and return the plate to the incubator. Be sure to gently shake the plate left to right and back to front to obtain even distribution of cells (do not swirl the dish as the cells will collect in the middle of the dish).
19. Incubate cells overnight to allow small colonies to attach.
20. The next day, change the medium with fresh Human Embryonic Stem Cell Growth Medium (pre-warmed to 37°C).
21. Refeed cells daily until it is time to split again.

Hints

Time to Change Medium

It is necessary to refeed Human Embryonic Stem Cells daily.

Time to Split Human Embryonic Stem Cells

1. Mouse Embryonic Fibroblast (MEF) feeder layer is two weeks old.
2. Colonies are too dense or too large.

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