OriCell Human Mesenchymal Stem Cells/GFP

Catalog No. HUXMA-01101

Instructions for Use

Materials Required (not supplied)

1. Trypsin-EDTA (Cat. No. TEDTA-10001-100）
2. Phosphate-Buffered Saline (1×PBS) (Cat. No. PBS-10001-500)
3. Human Mesenchymal Stem Cell Growth Medium (Cat. No. HUXMA-90011)

Thawing of Human Mesenchymal Stem Cells/GFP

1. Prepare 37°C water bath and pre-warm Human Mesenchymal Stem Cell Growth Medium to 37°C.
2. Add 9 mL of Human Mesenchymal Stem Cell Growth Medium to a 15 mL conical tube.
3. Remove the cryovial of Human Mesenchymal 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 Human Mesenchymal 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 this 1 mL of 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 2-3 mL of fresh Human Mesenchymal Stem Cell Growth Medium (pre-warmed to 37°C).
10. Gently re-suspend the cells in Human Mesenchymal Stem Cell Growth Medium.
11. Plate the cells into Two T25 flasks and add sufficient Human Mesenchymal 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 Human Mesenchymal Stem Cell Growth Medium (pre-warmed to 37°C).
14. Change the growth medium every three days thereafter.
15. When the cells are approximately 80 to 90% confluence, they can be dissociated with Trypsin-EDTA and passaged.

Changing Medium

1. Warm an appropriate amount of medium to 37°C in a sterile container. Remove the 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 the Human Mesenchymal Stem Cell Growth Medium, 1×PBS, Trypsin-EDTA solution to 37°C.
2. Carefully aspirate spent medium from the 80 to 90% confluent monolayer of Human Mesenchymal Stem Cells/GFP.
3. Add 1×PBS (6 mL for T75 flask, 3 mL for T25 flask). Be careful not to disturb the monolayer. Rinse the monolayer by gently rocking the flask back and forth.
4. Aspirate 1×PBS and discard.
5. Repeat the step 3-4 two or three times.
6. Add Trypsin-EDTA solution (1.5 mL for T75 flask, 0.5 mL for T25 flask). Gently rock the flask back and forth to ensure that the entire monolayer is covered with the Trypsin-EDTA solution. Allow the trypsinization to continue until the majority of the cells (approximately 80%) are rounded up. At this point, gently tap the side of the flask to release the majority of cells from the culture surface.

Note: Avoid leaving cells exposed to the trypsin longer than necessary. Care should also be taken that the cells not be forced to detach prematurely, as this may result in clumping.

7. After the cells are visibly detached, immediately add Human Mesenchymal Stem Cell Growth Medium (pre-warmed to 37°C) (6 mL for T75 flask, 3 mL for T25 flask) to neutralize the trypsinization.
8. Gently pipet the medium over the cells to dislodge and re-suspend the cells. Repeat 5-6 times until all the cells are dissociated from the flask and evenly dispersed into a single cell suspension.

Note: Care should be taken to avoid introducing bubble during pipeting.

9. Transfer the dissociated cells into a 15 mL conical tube.
10. Centrifuge at 250 g for 5 minutes to pellet the cells.
11. Carefully aspirate as much of the supernatant as possible.
12. Add 2 mL of Human Mesenchymal Stem Cell Growth Medium to the conical tube and re-suspend the cells thoroughly but gently.
13. Plate the cells into appropriate flasks. Human Mesenchymal Stem Cells/GFP can be split at 1:2 or other appropriate ratio.
14. Add sufficient medium.
15. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

Hints

Time to Change Medium

Although the cells do not reach 80 to 90% confluence, if the medium becomes acidic (the pH indicator in culture medium appears yellow), it is recommended that the medium be changed. In general, change the growth medium every three days.

Time to Subculture

When Human Mesenchymal Stem Cells/GFP reach 80 to 90% confluence, it is recommended that the cells be subcultured. Don't let Human Mesenchymal Stem Cells/GFP overgrow, or it will result in contact inhibition.