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MESENCHYMAL STEM CELL EXPANSION MEDIUM

CATALOG NUMBER: SCM015 **QUANTITY:** 500 mL

LOT NUMBER:

DESCRIPTION: Millipore's Mesenchymal Stem Cell Expansion Medium has been optimized and qualified for the growth and expansion of mesenchymal stem cells derived from human and rodent origins. Cells expanded in Mesenchymal Stem Cell Expansion Medium express the correct mesenchymal stem cell markers and are furthermore capable of differentiating into adipocytes and osteocytes.

PRESENTATION: Mesenchymal Stem Cell Expansion Medium is a proprietary formulation that contains fetal bovine serum. Sterility Testing: Negative

MATERIALS REQUIRED BUT NOT PROVIDED:

1. Cryopreserved Rat Mesenchymal Stem Cells (Catalog No. SCR027)
2. Human Mesenchymal Stem Cells, bone-marrow derived (Catalog No. SCR108)
3. Accutase™ Dissociation Solution (Catalog No. SCR005)

RELATED PRODUCTS:

1. Mesenchymal Stem Cell Characterization Kit (Catalog No. SCR018)
2. Mesenchymal Stem Cell Adipogenesis Kit (Catalog No. SCR020)
3. Mesenchymal Stem Cell Osteogenesis Kit (Catalog No. SCR028)
4. Mesenchymal Stem Cell Freezing Medium (Catalog No. SCR016)

THAWING CELLS:

1. Do not thaw the cells until the recommended medium and appropriate plasticware and/or glassware are on hand.
2. Remove the vial of mesenchymal stem cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of Mesenchymal Stem Cell Expansion Medium (pre-warmed at 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of medium at once to the cells. This may result in decreased cell viability due to osmotic shock.**
6. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT: Do not vortex the cells.**
7. Centrifuge the tube at 300 xg for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).

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9. Resuspend the cells in a total volume of 10 mL of Mesenchymal Stem Cell Expansion Medium (pre-warmed to 37°C).
10. Plate the cell mixture onto a 10-cm tissue culture plate.
11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.
12. The next day, exchange the medium with fresh Mesenchymal Stem Cell Expansion Medium (pre-warmed to 37°C). Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Accutase solution and passaged or alternatively frozen for later use.

SUBCULTURING:

1. Carefully remove the medium from the 10-cm tissue culture plate containing the confluent layer of mesenchymal stem cells.
2. Apply 3-5 mL of Accutase solution and incubate in a 37°C incubator for 3 minutes.
3. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.
4. Apply 5 mL of Mesenchymal Stem Cell Expansion Medium (pre-warmed to 37°C) to the plate.
5. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 xg for 2-3 minutes to pellet the cells.
7. Discard the supernatant
8. Apply 2 mL of Mesenchymal Stem Cell Expansion Medium to the conical tube and resuspend the cells thoroughly. IMPORTANT: Do not vortex.
9. Count the number of cells using a hemacytometer.
10. Plate the cells to the desired density into the appropriate flasks, plates or wells in Mesenchymal Stem Cell Expansion. We typically plate the cells at ~2 million cells per 10-cm plate or T75 flask.

STORAGE/HANDLING:

The Mesenchymal Stem Cell Expansion Medium should be stored at -20°C until ready to use. Upon thawing, the basal medium may be stored at 2-8°C for up to one month.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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