

MESENCHYMAL STEM CELL FREEZING MEDIUM (1X)

CATALOG NUMBER: SCM016

LOT NUMBER:

QUANTITY: 50 mL

DESCRIPTION: Mesenchymal Stem Cell Freezing Medium is qualified for use with mesenchymal stem cells of human and rodent (Cat. No. SCR027) origins that are cultured with Mesenchymal Stem Cell Expansion Medium (Cat. No. SCM015). The optimized formulation allows for consistent cryopreservation and high viability upon thawing and plating.

APPLICATIONS: Cryopreservation of human and rodent mesenchymal stem cell lines

PROTOCOL:

1. Thaw cell culture freezing medium completely and mix well by gently swirling bottle. Keep freezing medium on ice during use.
2. Cells to be frozen should be in late log phase growth.
3. Monolayers will need to be dissociated. After dissociation, cells are resuspended in Mesenchymal Stem Cell Expansion Medium (Cat. No. SCM015) and counted to determine viability and number.
4. Centrifuge cells at 300 x g for 3 min. Remove the medium above the pellet.
5. Resuspend the cells in Mesenchymal Stem Cell Freezing Medium at a concentration of $\sim 4 \times 10^6$ cells/mL. Freeze 1 mL of cells/vial. After the cells have been resuspended and aliquoted into appropriate cryogenic storage vials, they can be placed in a freezing container and the normal freeze down procedure should be started within five minutes.
6. Cells must be stored at or below -80°C . For long term storage the cells should be stored in ultra-low temperature freezer (-150°C), or in liquid nitrogen (-196°C).
7. Thawing of cryopreserved cells should be as follows:
 - a. Do not thaw the cells until the recommended medium and appropriate plasticware and/or glassware are on hand.
 - b. Thaw cells quickly in a 37°C water bath. **Important: Do not vortex the cells.**
 - c. Sterilize vial by rinsing with 70% ethanol.
 - d. 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.
 - e. 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.**
 - f. 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.**
 - g. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
 - h. Decant as much of the supernatant as possible
 - i. Resuspend the cells in a total volume of 10 mL of Mesenchymal Stem Cell Expansion Medium (pre-warmed at 37°C).
 - j. Plate the cell mixture onto a 10-cm tissue culture plate.
 - k. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.
 - l. 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.

- m. When the cells are approximately 80% confluent, they can be dissociated with Accutase[™] (Cat. No. SCR005) and passaged or alternatively frozen for later use.

PRESENTATION: Serum-containing formulation. Contains 10% DMSO.

**STORAGE/
HANDLING:** Store at -20°C. Refer to lot expiration date on label.

For research use only; not for use as a diagnostic.

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