hMPro™ Human Mesenchymal Progenitor Cells (Catalog#: HN60003)

FOR RESEARCH USE ONLY
COMMERCIAL USE PROHIBITED

Contents

- 1 vial of hMPro™ Human Mesenchymal Cells

Required but not Supplied

- ES qualified FBS
- α MEM basal medium
- L-Glutamine (200mM)

Optional but not Supplied

- Penicillin (5,000 U/ml)/Streptomycin (5,000 µg/ml)

Unpacking and Storage Instructions

- Cells must be moved from dry ice to liquid nitrogen IMMEDIATELY. Temperature fluctuations will have adverse effects on cell health and viability.
- When stored in the recommended storage conditions (liquid nitrogen), hMPro™ Human Mesenchymal Cells can remain stable in excess of 3 years.

Supplementing α MEM Basal Medium

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the amount indicated below to the basal medium with a pipette.

To make 100 mL of complete medium:	
α MEM basal medium (Invitrogen Cat# 12571)	89 mL
ES Qualified FBS (Hyclone Cat# SH30070.03E)	10 mL
L-Glutamine	1 mL
Penicillin/Streptomycin (optional)	1 mL

3. Supplemented medium should be stored at 2-8^{o}C, protected from light. The complete medium should be given a 2 week expiration date. Dispense the complete medium into aliquots to avoid repeated heating prior to each use

Cell Thawing Protocol

hMPro™ Human Mesenchymal Cells form adherent monolayer cultures when grown on cell culture plates. We recommend thawing your hMPro™ Human Mesenchymal Cells using the following protocol.

Required but not supplied:
Cell culture treated plates

To Plate the cells perform the following steps:

1. Do not thaw the cells until the recommended medium and appropriately coated plasticware and/or glassware are on hand.
2. Remove the vial 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. Breaking cells down to single cell suspensions will significantly increase cell death.

3. As soon as the cells are completely thawed, disinfect the outside of the vial with ethanol or isopropanol. 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 fully supplemented α MEM (pre-warmed to 37oC) 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 pipetting up and down twice. Be careful to not introduce any bubbles.

IMPORTANT: Do not vortex the cells. Breaking cells down to single cell suspensions will significantly increase cell death.

7. Centrifuge the tube at room temperature at 200 x g for 4 minutes to pellet the cells.
8. Aspirate as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 2 mLs of fully supplemented α MEM (pre-warmed to 37°C).
10. We recommend plating a vial of hMPro™ cells onto a 100 mm dish with a total of 10 mL of media.
11. Incubate the cells at 37°C in a 5% CO2 humidified incubator.
12. Exchange the medium with fresh fully supplemented α MEM medium (pre-warmed to 37°C) 24 hours post plating. Exchange with fresh medium every other day thereafter. Use caution not to dislodge the cells; do not pipette media directly onto the cells but rather onto the side of the culture dish.
13. Once the hMPro™ cells reach 80% - 85% confluence, they can be dissociated enzymatically and passaged. The cells should be maintained at an appropriate density at all times and thus a recommended plating value of 13,000 cells/cm^2.

Subculture of hMPro™ Cells

Required but not supplied:
0.25% Trypsin/EDTA
10% ES Qualified FBS in PBS +/+
PBS -/-
Cell culture treated plates

1. Once the hMPro™ cells reach 80% - 85% confluence, carefully remove the medium from the 100mm dish and rinse once with Phosphate Buffered Saline -/-.
2. Apply 5 mL fully supplemented medium (pre-warmed to 37°C) to the cells so that the cells can be harvested in fresh medium.
3. Using a 0.25% Trypsin/EDTA (pre-warmed to 37°C), enzymatically detach the cells from the dish by placing the dish on a slide warmer or an incubator at 37°C. Add enough trypsin to cover the surface of the plate. For a 100 mm plate, we recommend 2 mL of trypsin. Watch periodically with a microscope until the cells detach (approximately 3-5 minutes). It may be necessary to tap the flask to ensure detachment.
4. Plates should be observed to ensure that all cells have been removed. This is most easily accomplished by working under a dissection microscope within a laminar flow hood, but can also be achieved by frequent observation under a bright field or phase contrast microscope.
5. Add an equal volume of prepared 10% ES Qualified FBS in PBS +/+ solution to the plate to inactivate/dilute the trypsin (this can also be performed by adding an equal volume of fully supplemented α MEM medium)
6. Transfer the dissociated cells to a 15 mL conical tube. Inspect the plate to ensure that all the cells have been removed. Rinse the plate well with media and transfer the rinse to the 15 mL conical tube.
7. The cells can be centrifuged at 200 x g for 4 minutes in order to count the cells and replate at a specific concentration.
8. Plate the cells to the desired density into the appropriately flasks, plates or wells. We recommend keeping the cells at a density of 13,000 cells/cm^2.
9. Exchange the medium with fresh fully supplemented α MEM medium (pre-warmed to 37°C) 24 hours post plating. Exchange with fresh medium every other day thereafter. Use caution not to dislodge the cells; do not pipette media directly onto the cells but rather onto the side of the culture dish.

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