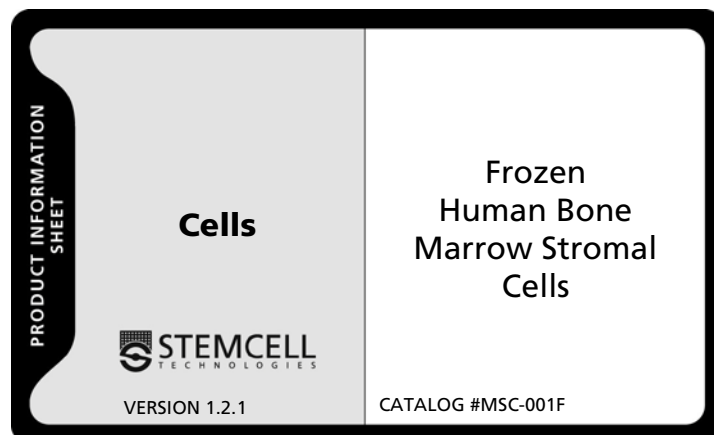


PRODUCT DESCRIPTION

Primary human marrow stromal cells (MSCs) have been cultured from adult bone marrow (BM) mononuclear cells (MNCs). These cells have fibroblast-like morphology. The cells are cryogenically preserved.

CATALOG #MSC-001F 0.75 million cells



SAMPLE COLLECTION AND PROCESSING

BM is drawn from the posterior iliac crest, 25 mL/site, from a maximum of 4 sites/donor. BM is diluted with PBS containing heparin (125 U/mL of BM).

MNCs are obtained by light density separation (1:2 dilution in PBS and centrifugation over Ficoll-Paque™). Red blood cells are lysed using ammonium chloride.

BM MNCs are cultured with prescreened FBS and modified medium for 10 – 14 days to obtain MSCs, which are frozen down immediately after the primary passage. The phenotype of these cells is assessed by flow cytometry.

NOTE: While these marrow stromal cells may exhibit mesenchymal stem cell phenotype, they have not been functionally tested in culture assays for multipotent differentiation capabilities.

PURITY

MSC purity is assessed to be greater than 90% expression of CD29, CD44, CD105, CD166 and less than 1% expression of CD14, CD34, CD45 by flow cytometric analysis. Please see the flow cytometry plot in the accompanying *Certificate of Analysis* for lot-specific purity.

FORMAT

Cryopreserved MSCs are frozen in a 1.8 mL solution of 50% IMDM, 40% FBS, and 10% DMSO (v/v; final concentrations).

STABILITY AND STORAGE

Cryopreserved samples are stable at -135°C or colder, or in liquid nitrogen for 6 months following date of receipt. Short-term storage of cells (<1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. Thawed samples must be used immediately.

Ficoll-Paque™ is a trademark of GE Healthcare.

THAWING INSTRUCTIONS

1. Warm desired medium in a 37°C water bath. Suggested medium: IMDM, DMEM, or RPMI-1640, all containing 10% FBS (v/v; final concentration).
2. Clean the outside of the frozen vial of cells with 70% alcohol before thawing.
3. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then re-tighten the cap.
4. Thaw the vial of frozen cells in a 37°C water bath. Remove the vial from the water bath immediately after it has thawed. Wipe the outside of the vial with 70% ethanol. **Do not vortex cells during thawing. It is important to work quickly from this point onwards to ensure high viability and recovery.**
5. Gently invert vial to mix cells.
6. In a biosafety hood, measure the volume of the cell suspension, for use in calculating the total starting cell concentration.
7. Aseptically remove a 10 µL aliquot of cells from the vial for counting. A separate person should proceed with **Counting Instructions**.

NOTE: This counting step is necessary to confirm the number of cells provided. Two people are required to carry out both the thawing and counting as these steps must be performed simultaneously.

8. Aseptically transfer the cell suspension to a 50 mL conical tube containing DNase I (Catalog #07900) to prevent cell clumping. For purified cells, a total of 100 µg of DNase I is recommended per mL of cell suspension. DNase I is not required if the cells will be used for subsequent DNA or RNA extraction.
9. Rinse the vial with 1 mL desired medium to recover remaining cells and slowly add this medium drop-wise to the cells in the 50 mL tube while gently swirling the tube. This should be done at a rate of approximately 1 drop every 5 seconds in order to ensure high viability.
10. Slowly add desired medium (from Step #1) to the cells until the final volume is 15 - 20 mL. Replace the cap and gently invert to mix.

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11. Centrifuge the cell suspension in a swinging bucket rotor in a benchtop centrifuge at 200 x g at room temperature for 15 minutes.
12. Carefully remove the supernatant with a pipette, leaving a small amount of medium behind to ensure cell pellet is not disturbed. Gently resuspend cell pellet by tapping the edge of the tube.
13. Fill the tube with 15 - 20 mL of desired medium, replace the cap and gently invert to mix.
14. Centrifuge the cell suspension at 200 x g at room temperature for 15 minutes.
15. Carefully remove supernatant with a pipette, leaving a small amount of medium behind to ensure that the cell pellet is not disturbed.
16. Gently flick the tube to resuspend the cell pellet and add the desired volume of medium to cells for further experiments.

NOTE: Cell losses should be expected during the thaw and wash steps. Cell loss may be up to 30%. Recovery rates differ due to individual variations in thawing techniques.

COUNTING INSTRUCTIONS

1. Clean coverslip and hemacytometer thoroughly with alcohol and dry with lens paper prior to using. Place coverslip on the hemacytometer so that it is centered over both chambers.
2. Take an aliquot of your sample to dilute for cell counting according to your laboratory standards as required (e.g. place 10 μL of cell suspension into 90 μL medium to achieve a 1:10 dilution).
3. Make a further 1:2 dilution of your cell suspension with Trypan Blue (Catalog #07050) to test viability by Trypan Blue dye exclusion (e.g. place 20 μL of 1:10 diluted cell suspension into 20 μL Trypan Blue).
4. Mix gently and let stand for 2 - 5 minutes.
5. Use a microhematocrit capillary tube or micropipette to fill chamber with cell suspension, being careful not to overfill or underfill.
6. Using a multichannel counter or two hand tally counters, score each viable (clear, bright, round, well-defined) and non-viable (blue, dull, damaged cell membranes) nucleated cell separately.
7. Count all cells in at least two of the major squares (nine x 1 mm² squares per chamber). Count opposite corner squares first and continue counting until a minimum of 100 nucleated cells have been scored. Keep a running total and establish an average number of cells/square.

NOTE: If there are <10 cells/square, a more concentrated cell suspension should be prepared. If there are >100 cells/square, a more diluted cell suspension should be prepared.

8. Determine the number of viable cells in your original sample using the following calculations.

NOTE: Each of the nine major squares of the hemacytometer, with coverslip in place, represents a total volume of 0.1 mm³ (equivalent to 10⁻⁴ cm³ or 10⁻⁴ mL).

Cells/mL = average count per square x dilution factor x 10⁴

Total Cells = cells/mL x original starting volume

% Viability = viable cells / (viable + nonviable) cells

Total Viable Cells = Total Cells x % viability

CULTURE INSTRUCTIONS

Please note that the culturability of these cells is not guaranteed under the Product Warranty.

For hints and tips on culturing, please contact techsupport@stemcell.com

WARNING

This product contains human material and must be treated as a potentially infectious and contaminated biological specimen, even if available serological reports are negative. Cells should be handled at Biosafety Level 2 (BSL-2) or higher. Universal handling precautions for biological samples should be used. For more information, please see your site Safety Officer or contact us at techsupport@stemcell.com.

PRODUCT WARRANTY

STEMCELL Technologies warrants primary cell products to meet the claimed product specifications, including viable cell number and purity, when the recommended protocols are followed. STEMCELL assures its cells to be viable and cell numbers recovered to be accurate, when handled exactly according to our instructions for thawing and counting. STEMCELL cannot guarantee biological function or any other properties associated with performance of cells in researchers' individual assay or culture systems.

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WARNING

We do not recommend the storage of frozen cell products in the liquid phase of a liquid nitrogen storage tank. Liquid can enter closed screw top cryovials, which then have the potential to explode due to internal pressure when removed from storage.

RECOMMENDED FROZEN CELL STORAGE CONDITIONS

For short-term storage (<1 month), store cells in -80°C freezer.

For long-term storage (>1 month), store in the vapor phase of a liquid nitrogen storage tank.

STORAGE PRECAUTIONS

WARNING: We do not recommend the storage of frozen cell products in the liquid phase of liquid nitrogen (LN₂). Liquid can enter closed screw top cryovials, which then have the potential to explode when removed from storage.

Our warranty does not cover any losses or damages of any kind due to storage of products in the liquid phase of LN₂.

Laboratory personnel should use extreme caution when storing samples in LN₂. LN₂ storage consists of a liquid phase and a gaseous phase. If cryovials are immersed in the liquid phase, LN₂ can enter the closed screw-top cryovials during storage. The cryovial may then explode when it is removed from storage due to the vaporization and expansion (700x expansion ratio) of the liquid nitrogen inside the cryovial.

HEALTH HAZARDS OF LIQUID NITROGEN

Liquid nitrogen has a 700x expansion ratio, which may cause physical hazards and injuries due to the explosion of cryovials, containers, equipment, or other devices. Extensive tissue damage or burns can result from exposure to LN₂ or cold nitrogen vapors. Asphyxiation may result from the displacement of oxygen in the air with nitrogen to levels where there is insufficient oxygen. Inhalation of oxygen deficient air can cause dizziness, nausea, vomiting, loss of consciousness, and death.

PERSONAL PROTECTIVE EQUIPMENT

The following personal protective equipment is recommended when handling or using LN₂:

Cryo gloves/Waterproof thermal insulated gloves

- Hands should be protected with waterproof thermal insulated gloves that can be quickly removed if LN₂ is spilled on them. These gloves are not intended for submersing hands into LN₂.

Clothing

- Body must be protected with pants, lab coats, and closed-toe shoes.

Face Shield

- Eyes are sensitive to the extreme cold of LN₂ and its vapors. Over-pressurization may result in the explosion of improperly stored cryovials. Chemical splash face shields should be used when handling LN₂ and when handling cryovials and other sealed containers that have been stored in LN₂.

The handling of cryovials inside of Biological Safety Cabinets (with the sash lowered) will further reduce the risk of injury from explosions caused by excess pressure within the vial. We recommend that excess pressure be relieved by briefly opening the cap of the cryovial a quarter turn before resealing. This should be done inside a Biological Safety Cabinet.