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Thawing Human MSCs in NutriStem® MSC Medium and Human Platelet Lysate

Introduction

Human mesenchymal stem/stromal cells (MSCs) are promising tools for a range of applications in both research and translational studies, including direct differentiation and tissue regeneration, immunomodulation, and cancer research. MSCs can be cultured in vitro for multiple passages while maintaining their immunomodulatory profile and multipotent differentiation potential, enabling the production of sufficient cell numbers required for many cell-based therapeutic strategies.

Human MSCs show exceptional recovery and expansion when thawed and cultured in NutriStem® MSC Medium supplemented with 5% PLTGold® Human Platelet Lysate. MSCs previously cultured in other serum-containing or serum-free media can be efficiently thawed directly into NutriStem® MSC Medium and 5% PLTGold® Human Platelet Lysate. Because the thawing procedure can be stressful to frozen cells, good technique and highly supportive culture media is essential to maximizing recovery. Working gently and quickly to thaw and plate cells will improve cell survival after cryopreservation.

Required Materials

Reagent	Source	Cat. No.
Human BM-MSCs, Frozen	BI-USA	BMMSC001C (or) BMMSC002C
NutriStem® MSC Medium*	BI-USA	05-200-1A-KT
PLTGold® Human Platelet Lysate*	BI-USA	PLTGOLD100 (or) PLTGOLD27
Trypan Blue Solution (5 mg/mL)	BI-USA	03-102-1B

Hemocytometer and Cover Slips

* Research and Clinical Grade solutions available

Important Notes

- Thawing must be performed as quickly as possible to improve cell viability and recovery from cryopreservation. Have all media prepared and equipment and accessible prior to beginning the thawing protocol.
- Warm an aliquot of complete MSC medium prior to beginning the thawing protocol. Warm enough media to thaw, count, and plate cells for culture.
- Trypan Blue Solution (working concentration = 5 mg/mL) is commonly used to distinguish viable from non-viable cells.
- Human MSCs, like all products of human origin, must be handled following universal precautions at Biosafety Level 2 or higher.
- Cryovials stored in liquid nitrogen present a risk of rupturing with a dramatic temperature change. Wear appropriate personal protective equipment when handling vials stored in liquid nitrogen.

Reagent Storage and Notes

NutriStem® MSC Medium

- NutriStem® MSC Medium contains a basal medium and supplement mix. Adding the supplement mix can further increase MSC proliferation and expansion rates. For certain applications, the addition of the supplement mix may be optional.
- Store the basal medium at 4°C, protected from light.
- The basal medium contains stable L-alanyl-L-glutamine. No additional L-glutamine is necessary.
- Store the supplement mix at -20°C, protected from light.
- The frozen supplement mix should be thawed at room temperature or at 4°C before use.
- If not using immediately, prepare single-use aliquots of the supplement mix from the stock solution and re-freeze at -20°C. Avoid repeated freeze/thaw cycles (up to two times).

PLTGold® Human Platelet Lysate

- Thaw frozen PLTGold® in a 37°C water bath, protected from light. Mix gently but thoroughly once thawed.
- If not using the entire thawed solution of PLTGold® immediately, prepare single-use aliquots from the stock solution and re-freeze at -20°C.
- Avoid exposing PLTGold® to repeated temperature changes or freeze/thaw cycles. Long-term storage of PLTGold® at 4°C is not recommended.
- PLTGold® Human Platelet Lysate does not require the addition of heparin or any anti-coagulant for use.

Culture Medium Preparation and Storage

To prepare 100 mL of complete MSC medium, aseptically combine the following components:

NutriStem® MSC Basal Medium	100 mL
NutriStem® MSC Supplement Mix	0.6 mL
PLTGold® Human Platelet Lysate	5.5 mL

To prepare 500 mL of complete MSC medium, aseptically combine the following components:

NutriStem® MSC Basal Medium	500 mL
NutriStem® MSC Supplement Mix	3.0 mL
PLTGold® Human Platelet Lysate	26 mL

Store the complete MSC culture medium at 4°C, protected from light, for up to 2 weeks.

Protocol for Use

The following protocol outlines the steps to effectively thaw one vial of cryopreserved MSCs. For best results, cells should be thawed, plated, and placed in an incubator for culture as quickly as possible. MSCs previously cultured in other serum-free or serum-containing media can be efficiently thawed directly into NutriStem® MSC Medium supplemented with 5% PLTGold® Human Platelet Lysate. Follow aseptic techniques and perform cell culture work in a Class II biological safety cabinet.

Thaw MSCs

Cells are often frozen in a medium containing the cryoprotective agent DMSO. Although DMSO protects the cells while cryopreserved, it is toxic to cells at room temperature, even for a very short period of time. Work quickly but gently when thawing cells to maximize viability and survival post-thaw.

1. Warm an aliquot of complete MSC culture medium at 37°C prior to thawing cells. Warm enough medium to thaw, count, and plate cells for culture.
2. Remove the cryovial from liquid nitrogen storage and place under dry ice until ready to thaw.

   Note: For best results, thaw vial immediately upon removing from liquid nitrogen storage.
3. To thaw, immerse the cryovial in a 37°C water bath and swirl the vial gently for approximately 60 seconds, or until only a small ice crystal remains.

   Note: Avoid submerging the cap of the cryovial in the water bath to prevent potential contamination.
4. Sterilize the cryovial with 70% ethanol and transport to a biosafety cabinet.
5. Transfer the cells from in the cryovial directly to the bottom of a 15 mL conical tube.
6. Slowly add 6 mL of complete MSC medium to the conical tube containing the cell suspension to dilute the DMSO.

   Note: Add medium slowly and dropwise to the cell solution to prevent osmotic damage to the cells.
7. Centrifuge the cell suspension at 200 x g for 5 minutes at room temperature. If possible, set centrifuge settings to a gradual start and slow break.
8. Carefully remove the supernatant and gently resuspend the cell pellet in 2 mL of complete MSC medium.

   Note: Resuspend the cells using gentle pipetting – do not vortex.
9. Note the total cell suspension volume in the 15 mL conical tube (Starting Volume).

Count Cells and Assess Viability

Trypan Blue is used at a working concentration of 5 mg/mL. In solution with Trypan Blue, viable cells will appear clear, round, and bright when viewed on a hemocytometer under a microscope. Non-viable cells will take up the dye and appear blue, dull, and may be irregularly shaped. For accurate viability assessment, do not allow the cells to sit for an extended period of time after staining with Trypan Blue, as eventually viable as well as non-viable cells may begin to absorb the dye.

One square of a hemocytometer = 100 nL

10. Clean a hemocytometer and glass coverslip with 70% ethanol to remove any dust particles, and wipe with lens paper.
11. Gently pipet the cell suspension prepared in step 8 to evenly distribute the cells, and transfer 20 µL of the well-mixed suspension to a 1.5 mL microfuge tube.
12. Add 20 µL of Trypan Blue to the same microfuge tube containing the cell suspension and mix well to create a 1:1 dilution. (Dilution Factor = 2)
13. Load the Trypan Blue/cell solution on each side of a prepared hemocytometer by capillary action, being careful not to over-or under-fill the chamber.
14. Count viable cells and non-viable cells in all 4 corner squares on each side of the hemocytometer under a microscope at 10X magnification.

    Note: If cell count is higher than 100 cells per square, repeat steps 10 through 14 with a more dilute cell solution. For example, combine 20 µL of well-mixed cell suspension with 60 µL of Trypan Blue. (Dilution Factor = 4)

15. Determine the number of total viable cells in the cell suspension.

% Viability = $\frac{\text{Number of Viable Cells}}{\text{All Cells}}$

Cell Concentration (cells/mL) = (Mean Viable Cells per Square) x (Dilution Factor) x (10^4)

Total Cell Count = (Cell Concentration) x (Starting Volume)

Total Viable Cell Count = (Total Cell Count) x (% Viability)

Plate MSCs for Culture

Plating cells at a high density can improve recovery and survival from a thaw. For best results, plate MSCs initially at a density of 5 x 10^3 cells/cm^2 from a thaw following the guidelines below. Do not centrifuge cells to concentrate the suspension prior to plating.

16. Plate the cells at a seeding density of 5 x 10^3 viable cells/cm^2 according to Figure 1 below.
17. Immediately transfer the cells to a 37°C humidified incubator, and gently rock the plate side to side to evenly distribute the cells across the surface of the culture dish.
18. Incubate the cells overnight at 37°C and 5% CO_2.
19. Observe cells daily to monitor cell health, proliferation, and confluence. Perform a complete medium change every other day or as needed between passages.

Refer to the protocol **Passaging MSCs using NutriStem® MSC Medium and Human Platelet Lysate** for more information on MSC culture and expansion.

Figure 1. Appropriate plating densities for human MSCs post-thaw. Cell numbers are based on a plating cell density of 5 x 10^3 cells/cm^2 and calculated using the surface area of the culture vessel.

Culture Vessel	Surface Area	Recommended MSC Plating Density at 5 x 10^3 cells/cm^2
6-well Plate	9.6 cm^2 per well	4.8 x 10^4 cells per well
12-well Plate	3.8 cm^2 per well	1.9 x 10^4 cells per well
24-well Plate	2 cm^2 per well	1.0 x 10^4 cells per well
35 mm Dish	9.6 cm^2	4.8 x 10^4 cells
T25 Flask	25 cm^2	1.25 x 10^5 cells

For additional product or technical information, please visit the Biological Industries USA web site at www.bioindusa.com, email techsupport@bioindusa.com, or call customer service at 1-860-316-2702.