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Measuring Viability and Plating Fresh BM-MSCs

Introduction

This cell count and viability assessment protocol is intended to confirm the quality of fresh cells after shipment and should be performed immediately upon receipt of cells. After determining the number of viable cells in the culture, fresh bone marrow-derived mesenchymal stem/stromal cells (BM-MSCs) should be then plated in appropriate MSC culture conditions for further maintenance and expansion.

Human BM-MSCs show exceptional proliferation and expansion in NutriStem® MSC Medium supplemented with 5% human platelet lysate, however, cells may also be cultured in other supportive MSC media.

Required Materials

Reagent	Source	Cat. No.
StemExpress® Human BM-MSCs, Fresh	BI-USA	BMMSC001F BMMSC002F
Trypan Blue	BI-USA	03-102-1B
NutriStem® MSC Medium	BI-USA	05-200-1A-KT
PLTGold® Human Platelet Lysate	BI-USA	PLTGOLD100GMP PLTGOLD100R
Hemocytometer & Cover Slips		

Important Notes

- Count cells prior to washing. Cell loss during wash steps is expected and may result in 10 to 15% decrease in cell number per wash.
- Fresh cells must be processed, counted, and cultured immediately upon receipt.
- Purity of cells can also be assessed by staining the cells with appropriate antibodies via flow cytometry.
    - For Human BM-MSCs, appropriate positive antibody markers are CD73, CD90, and CD105
    - For Human BM-MSCs, appropriate negative antibody markers are CD14, CD34, and CD45
- Human BM-MSCs, like all products of human origin, must be handled following universal precautions at Biosafety Level 2 or higher.
- Refer to the protocol Passaging MSCs using NutriStem® MSC Medium and Human Platelet Lysate for more information on MSC culture.

Protocol for Use

The following protocol outlines the routine steps to measure viability of fresh cell cultures after shipment. For best results, cells should be processed and plated for culture immediately upon receipt. Follow aseptic techniques and perform cell culture work in a Class II biological safety cabinet.

Cell Counts and Assess Viability

In a solution with Trypan Blue, viable cells will appear clear, round, and bright when viewed on a hemocytometer under a microscope. Non-viable cells will appear blue, dull, and may be irregularly shaped.

One square of a hemocytometer = 100 nL

1. Clean a hemocytometer and glass coverslip with 70% ethanol to remove any dust particles, and wipe with lens paper.
2. Transfer the cells from the shipping vial to a sterile 15 mL conical tube.
3. Rinse the vial with 1 mL of MSC medium to collect any remaining cells, and transfer to the same 15 mL conical tube.
4. Gently pipet the cell suspension to mix cells and measure the total volume. (Starting Volume)
5. After proper resuspension, transfer 20 µL of the cell suspension to a small Eppendorf tube.
6. Add 20 µL of Trypan Blue to the same Eppendorf tube containing the cell suspension and mix well to create a 1-in-2 dilution. (Dilution Factor = 2)
7. Load the Trypan Blue/cell solution on each side of a prepared hemocytometer, being careful not to over-or under-fill the chamber.
8. Count viable and non-viable cells in all 4 corner squares on each side of the hemocytometer.

   Note: If cell count is higher than 100 cells per square, repeat steps 4 through 8 with a more dilute cell suspension. For example, combine 20 µL of well-mixed cell suspension with 80 µL of Trypan Blue. (Dilution Factor = 5)
9. 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

Plate cells in new culture dishes immediately after assessing viability. Human BM-MSCs show exceptional proliferation and expansion in NutriStem® MSC Medium supplemented with 5% human platelet lysate, but cells may also be cultured in other supportive MSC media.

10. Briefly warm a sufficient amount of complete MSC culture medium at 37°C.
11. Plate the cells at a seeding density of 2 x 10^3 to 5 x 10^3 viable cells per cm^2 and rock the dish gently to distribute the cells evenly across the surface area.
12. Incubate the cells overnight at 37°C and 5% CO_2.
13. Observe cells daily to monitor cell health, proliferation, and confluence. Perform a complete medium change every other day or as needed between passages.

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 1-860-269-0596.