

Technical Notes—How to Passage and Subculture— Monocyte-Derived Macrophages

General Media Requirements

Monocyte Derived Macrophages (MDM) will mature if cultured with Macrophage Basal Medium (Cat. No. PB-MDM-002) in conjunction with Macrophage Stimulatory Supplement (Cat. No. PB-MDM-003).

Types of Culture Vessels

- 96 well plate
- 35mm treated culture dish
- 100mm treated culture dish
- T25 flask
- T75 flask

Thawing and Culturing Cells

1. Calculate the number of culture vessels needed. The recommended seeding density for AllCells MDM is 10×10^4 cells/cm². For example, one vial of AllCells MDM (1.5×10^6 /vial) would be sufficient for six T25 flask or two 100mm dish.
2. Prepare the culture media: Add 2mM concentration of L-glutamine, 1% of the antibiotic-antimycotic stock solution and the entire contents of 50ml AllCells Stimulatory supplement (Cat. No. PB-MDM-003) to 450ml of AllCells Basal Medium (Cat. No. PB-MDM-002).
3. Wipe the frozen vial with 70% alcohol before thawing.
4. In a biosafety hood, briefly twist the cap a quarter-turn to relieve pressure, then retighten the cap.
5. In a 37°C water bath, quickly thaw the vial. Be careful not to submerge the entire vial. Do not remove the vial until a tiny ice-crystal is left.
6. Wipe the outside of the vial with 70% alcohol.
7. Count cells by taking 10 μ l from the vial to mix with 10 μ l of trypan blue solution. Count cells with a hemacytometer .

N = # of cells counted on all 4 squares of a hemacytometer
 d = dilution factor

Equation for Cell Count:

$$\# \text{ of cells/ vial} = N/4 \times 2 \times d \times \text{ml} = \text{ } 10^4$$

Equation for Viability:

$$\# \text{ of cells excluded by trypan blue/ \# total number of cells} \times 100\% = \text{ } \%$$

**This is a very important step to determine if the cell viability number matches what AllCells claims.

8. Initiate the culture. Resuspend and dispense 10,000 cells/cm² from the vial into the culture vessel.
9. Add the desired volume of prepared culture medium to the vessel.
10. Gently rock the culture vessel to evenly distribute the cells and place in 5% CO₂ incubator.

Note: It is not necessary to wash the cells because this action is more damaging than the effects of DMSO residue in the culture.

Maintaining Cell Culture

1. Change the growth medium the day after seeding and every other day thereafter.
2. In a sterile container, warm an appropriate amount of medium to 37°C. Remove the medium and replace it with the warmed, fresh medium
3. Return the flask to the incubator.

Note: Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer only the required volume to a sterile secondary container.