Ossium Inventa
5742 W. 74th St.
Indianapolis, IN 46278
United States
+1 (833) 4OSSIUM
products@ossiuminventa.com

Thawing & Passaging Human Bone Marrow Derived MSC

For Nonclinical Use Only, Not for Diagnostic or Therapeutic Use

Product Description

Ossium Inventa's human bone marrow derived mesenchymal stem cells (MSC) are aseptically obtained from vertebral bones recovered from deceased organ donors. The passaged MSC are cryopreserved with DMSO and human serum albumin. Ossium Inventa MSC are for nonclinical use only and should not be used in diagnostic or therapeutic applications.

For donor details and test results, refer to the lot-specific Certificate of Analysis.

Storage

Freeze cryopreserved Ossium Inventa MSC immediately upon receipt. For short-term storage, store at or below -86 ºC. For long-term storage, store in liquid nitrogen or vapor-phase liquid nitrogen.

Instructions for Use

Thawing & Plating

1.1. Follow biosafety level 2 procedures and universal precautions when handling these cells.
1.2. Ensure a water bath is warmed to 37 ºC.
1.3. Remove the cell vial from storage and quickly thaw in a 37 ºC water bath by gently shaking, taking care to not submerge the cap. Remove the vial when the last visible ice is just melted. NOTE: Work quickly to ensure high cell viability.
1.4. Wipe the outside of the vial with 70% ethanol or isopropanol and place in a biosafety cabinet. Work aseptically in the following steps.
1.5. Transfer thawed cells to a tube that can hold 3x the starting volume. Dilute the cells with 2x the volume of PBS. Mix well.
1.6. Centrifuge the diluted cells at 300×g for 5 minutes. Aspirate the supernatant, taking care to not disturb the cell pellet.
1.7. Gently flick the bottom of the tube to break up the pellet. Resuspend the cells in an appropriate medium to support MSC culture, gently pipetting up and down to mix the cells. Avoid introducing air bubbles.
1.8. Seed the cells at or around a density of 4,000 cells/cm^2 in a tissue culture plate or flask with an appropriate volume of medium. Gently rock the culture vessel in a figure-8 motion to ensure even distribution of cells. Example: For a T225 flask, use 45 mL of medium and seed 9×10^5 live cells.
1.9. Refeed cells every three to four days by aspirating the spent medium and replacing it with an equal volume of warmed, fresh medium.
1.10. Passage cells when the cells reach >75% confluency (approximately five to six days of culture). Cells must be harvested by day 7 regardless of confluency.

Ossium Inventa
5742 W. 74th St.
Indianapolis, IN 46278
United States
+1 (833) 4OSSIUM
products@ossiuminventa.com

Thawing & Passaging Human Bone Marrow Derived MSC

For Nonclinical Use Only, Not for Diagnostic or Therapeutic Use

Passaging

2.1. Follow biosafety level 2 procedures and universal precautions when handling these cells.

2.2. Warm 0.25% Trypsin-EDTA and MSC culture medium to 37 ºC.

2.3. Wipe the outside of all reagents and supplies with 70% ethanol or isopropanol and place in a biosafety cabinet. Work aseptically in the following steps.

2.4. Pipette the spent medium from the culture vessel (i.e., plate or flask) into a sterile container.

2.5. Transfer an appropriate volume of trypsin into the culture vessel. Gently tilt to cover the entire culture surface. Incubate at 37 ºC and 5% CO_2 for 5 minutes.

2.6. Verify that the cells have lifted from the culture surface via a microscope. Lifted cells appear as small floating spheres. If cells are still adhered, try one of the following:

   a. Gently tap your palm against the side of the culture vessel.

   b. Place the culture vessel back into the incubator for up to 3 minutes.

2.7. Once cells have lifted, place the culture vessel back into the biosafety cabinet.

2.8. Transfer the collected spent medium back into the culture vessel to neutralize the trypsin.

2.9. Transfer the full volume from the culture vessel into a tube. Rinse the culture surface with an appropriate volume of PBS. Transfer the PBS into the same tube.

2.10. Centrifuge the tube at 300×g for 5 minutes. Aspirate the supernatant, taking care to not disturb the cell pellet.

2.11. Gently flick the bottom of the tube to break up the pellet. Resuspend the pellet in culture medium, gently pipetting up and down to mix the cells. Avoid introducing air bubbles.

2.12. Seed the cells in a new culture vessel at or around a density of 4,000 cells/cm^2 with an appropriate volume of medium. Gently rock the culture vessel in a figure-8 motion to ensure even distribution of cells. Example: For a T225 flask, use 45 mL of medium and seed 9×10^5 live cells.

2.13. Refeed cells every three to four days by aspirating the spent medium and replacing it with an equal volume of warmed, fresh medium.

2.14. Passage cells when the cells reach >75% confluency (approximately five to six days of culture). Cells must be harvested by day 7 regardless of confluency.

Notes

It is not recommended to refreeze Ossium Inventa MSC after thawing.

It is recommended to use Ossium Inventa MSC before or at the fifth passage.