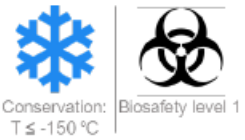
Human Bone Marrow Mesenchymal Stem Cells, Cryopreserved, 1.10^6 cells

Product sheet, catalog n° CTICC.IMBM.3

General Information

- **Organism:** Human (*Homo sapiens*)
- **Tissue:** Bone Marrow
- **Cell Type:** Bone Marrow Mesenchymal Stem Cells from single donor
- **Gender:** Male or Female (see Certificate of Analysis)
- **Age:** 18 to 99 years old (see Certificate of Analysis)
- **Donor type:** (see Certificate of Analysis)



Cell Characteristics

- **Cell properties:** Adherent
- **Morphology:** Adherent, spindle-shaped bipolar, fibroblast-like morphology growing as monolayer.
- **Isolation:** Enzymatic dissociation.
- **Cell passage**: (see Certificate of Analysis).
- **Cell viability:** Minimum 70% viability when thawed from cryopreservation*
- **Cell conditioning:** 1M viable cells before cryopreservation*
- **Seeding density**: 6000 to 10000 cells per cm^2
- **Cryopreservation medium:** Frozen with 90% serum-free cryopreservation medium + 10% DMSO
- **Storage condition:** Liquid nitrogen vapour phase
- **Batch specific information**: (see Certificate of Analysis)

* Thawing of the cell products may induce up to 30% cell loss depending on the operator's experience and thawing procedures

Safety and Quality Control

- **Biosafety level:** 1
- **Contamination:** Use mandatory laboratory protection and handle with care tissues and cells derived from human samples to avoid any contamination of the operator
- **Viral testing:** Negative for HIV, HBV, HCV
- **Sterility testing :** Negative for mycoplasma, bacteria and yeasts

Handling upon delivery and storage

- Check that the containers are intact and free of damage
- If not used immediately, place the vials at -150°C or below upon delivery

Thawing and culturing procedure for frozen cells

Note : Collagen pre-coated flask should be used for proper cell adhesion

1 - Culture cells in 0,15 to 0,25 ml per cm^2 of medium to the culture vessel.

2 - Add 14 ml of PBS solution to a 15 ml conical tube, and warm in a water bath to 37 °C.

3 - Thaw cryovial by swirling in a water bath at 37 °C. Stop when half of the content is thawed. Bring the cryovial and the tube containing warmed PBS under the Biological Safety Cabinet in sterile condition.

4 - Open the cryovial and without touching the remaining ice, dilute the content of the cryovial with 500 µL of warm PBS, transfer the thawed solution into a new 15 ml conical tube.
5 - Repeat Step 4 until all the content of the cryovial is thawed and wash the cryovial several times with warm PBS. Fill the tube containing the cell suspension up to 14 mL using the remaining warm PBS.
6 - Spin the tube at 300 g for 10 minutes to pellet the cells.
7 - Resuspend the cells in the appropriate volume of recommended medium.
8 - Seed the cells in collagen coated culture vessel at the recommended seeding density.
9 - Incubate at 37 °C, 5% CO_2 atmosphere, 95% humidity.
10 - After 24 h incubation, if most of the cells are attached to the vessel the medium can be changed to remove cell debris.
11 - Continue to incubate and perform medium renewal two to three times/week.

Subculturing

Note : Collagen pre-coated flask should be used for proper cell adhesion
1- Cells will stop growing when confluent.
2 - Preheat TrypLE (non-toxic for cell - trypsin substitute).
3 - Remove the medium from the flask and wash the cells quickly with PBS.
4 - Add 0,1 to 0,2 mL per cm^2 of TrypLE and incubate for 6 to 10 min at 37 °C in the incubator.
5 - Finish detaching the cells by flushing the flask several times with a serological pipette, remove the cells from the flask and wash the flask with PBS several times to harvest all remaining cells.
6 - Collect all flask content in a 15 mL conical tube and centrifuge at 300 g, 10 minutes to pellet the cells.
7 - Remove the supernatant and resuspend the pellet in recommended medium.
8 - Seed the cells in collagen coated culture vessel at the recommended seeding density.
9 - Incubate at 37 °C, 5% CO_2 atmosphere, 95% humidity.
10 - After 24 hours of incubation, if the cells have attached, change the medium to remove debris.
11 - Incubate and perform medium renewal two to three times/week.

Associated products

- **CTICC.IMBM.1:** Human Bone Marrow Mononucleated Cells, Cryopreserved, 1.10^6 cells
- **CTICC.IMBM.2:** Fresh Human Bone Marrow, 2 to 3mL

Provisions

- Cells and tissues are intended for **research use only** and shall not be used for human trials, animal trials, or diagnostics
- **Consent:** the original tissues have been obtained after informed consent of the patient under the provisions required by French Law
- **Primary Human cells** are not immortalised cell lines and may not be continually subcultured