

Thawing of Cryopreserved Ossium Inventa CD34+ Cells RUO

For Nonclinical Use Only, Not for Diagnostic or Therapeutic Use

Product Description

Prior to selection of Ossium Inventa CD34+ Cells RUO, Human Whole Bone Marrow (WBM) RUO is aseptically recovered from vertebral bones of organ donors via proprietary methods at Ossium Health's production facility. All donors are consented for recovery. CD34+ cells are aseptically selected from the recovered WBM RUO by positive immune separation. The selected CD34+ cells are analyzed for purity and viability by flow cytometry. Cells are adjusted to a final concentration in buffer containing 5% DMSO for cryopreservation.

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

Storage

Store cryopreserved Ossium Inventa CD34+ Cells RUO immediately upon receipt. For short-term storage, store at -86 °C. For long-term storage, store in liquid nitrogen or vapor-phase liquid nitrogen.

Instructions for Use

1. Follow biosafety level 2 procedures and universal precautions when handling the cells.
2. Ensure a water bath is warmed to 37 ± 1 °C prior to performing the thaw protocol. Work quickly to ensure cell viability.
3. Obtain the cryovial containing the cryopreserved CD34+ cells from frozen storage (-86C for short-term or LN2 for long-term) and place it into the 37 °C water bath immediately. Thaw cells in water bath by gently shaking the vial.
4. Remove the vial from the water bath when only a sliver of ice remains. Clean the outside of the vial with 70% ethanol and transfer the cryovial into a BSC. Do not vortex cells.
5. Pipette up and down gently.
6. Remove a 20 µL aliquot of cells and place on ice for later counting.
 - a. We recommend counting the cells before they have been transferred or washed to confirm the number of cells received. A manual count is recommended in parallel with automated counting to account for cytometer variability.
7. Pipette cells into a 15 mL conical centrifuge tube. We recommend using a centrifuge tube with a hydrophilic surface to prevent cells sticking to the walls.
8. Rinse the vial with 1 mL of medium and add to the 15 mL conical tube.
 - a. The type of media depends on customer's downstream application. Ossium routinely uses Plasma-Lyte containing 2.5% HSA. Protein-containing media are recommended to prevent cell clumping and sticking.
9. Add an additional 9 mL of media to the 15 mL conical tube. (See step 8a)
10. Pipette up and down gently.
11. Centrifuge the cell suspension at 300 to $400 \times g$ for 10 minutes at room temperature (15 to 25 °C).
12. Carefully remove the supernatant (from step 11) with a pipette without disturbing the cell pellet. Resuspend the cell pellet by gently flicking the tube.
13. Add 1 mL of medium and pipette gently. Cells are now ready for use in downstream applications.