Thawing Cryopreserved Primary Cells

Overview

When thawing cryopreserved cells, proper technique and handling ensures optimal viability, recovery, and functionality of the cells for downstream applications. This protocol describes a method for thawing cryopreserved human peripheral blood cells or subsets.

Materials

1. Human primary blood mononuclear cells (cryopreserved)
2. RPMI with L-Glutamine and 10% FBS
3. 70% EtOH
4. 15mL tubes (Falcon)
5. Pipets and pipet dispenser
6. 37℃ water bath
7. CO2 incubator
8. Centrifuge

Protocol

1. Warm water bath to 37℃, and ensure RPMI is warmed to 37℃.
2. When removing frozen cells from liquid nitrogen storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
3. Place cryopreserved cell vial in 37℃ water bath. Submerge cryovial halfway and thaw for approximately 2 minutes. Gently swirl vial.
4. Examine vial, continue 37℃ thaw until ice just before last ice crystal has melted. Do not allow vial to warm to greater than 10℃.
5. Transfer vial to a biosafety cabinet and wipe the outside of the vial with 70% ethanol or isopropanol.
6. Pour thawed cells into 15 mL conical tube containing 5mL of RPMI that has been pre-warmed to 37℃. Do not use a pipet for this step.
7. Rinse cryovial with 2 mL pre-warmed RPM using a pipet. Pour cells into 15 mL conical tube.
8. Incubate cells for 5 minutes at 37℃.
9. Centrifuge cell suspension at 260 x g at 20℃ (room temp) for 5 min, low brake.
10. Pour off the supernatant.
11. Add 2 mL pre-warmed RPMI using a pipet. Mix by flicking tube (avoid pipetting).
12. Incubate cell suspension for 1 hour to overnight in a 37℃ CO2 incubator. Leave cap loose so gas transfer can occur.
13. Cells are now ready for use in downstream applications.