

KHOS-NP | 300235

Thawing and Culturing Cells

1. **Thawing:** Thaw the vial rapidly in a 37°C water bath. Do not allow the cells to remain at room temperature for more than 5 minutes.
2. **Resuspension:** Resuspend the cells in 1 mL of pre-warmed complete medium. Gently mix by pipetting up and down. Do not vortex.
3. **Seeding:** Seed the cells into a 25 cm² flask containing 50 mL of pre-warmed complete medium. Add the cells to the flask and gently mix.
4. **Medium Change:** After 24 hours, replace the medium with fresh complete medium to remove any dead cells.
5. **Passaging:** When cells reach 80-90% confluency, passage them into a new flask. Use 15 mL of trypsin-EDTA solution for 15 minutes at 37°C. Add 5 mL of neutralizing medium to stop the reaction.
6. **Counting:** Count the cells using a hemocytometer. Seed approximately 3 x 10⁵ cells per flask.
7. **Medium:** Use DMEM supplemented with 10% FBS for initial growth. Switch to 5% FBS for expansion.
8. **Storage:** For long-term storage, harvest cells and resuspend in freezing medium. Store at -80°C.

Incubation Atmosphere

37°C, 5% CO₂, humidified

Flask Coating

Flasks are pre-coated with poly-L-lysine. No additional coating is required.

Freezing Procedure

Resuspend cells in freezing medium. Aliquot into 1 mL vials. Freeze at -80°C.

Shipping Conditions

Store at -80°C. Ship on dry ice in a cool box.

Storage Conditions

Store at -150°C for up to 196 months.

Genotype / Phenotype / HLA

Sterility

Cells are tested for mycoplasma contamination. PCR testing is available. All reagents are sterile.