





**Sf9 | 604328**

**Thawing and Culturing Cells**

1. Thaw vials rapidly in a 37°C water bath. Do not vortex. Remove vial and centrifuge at 300 x g for 5 minutes. Transfer cells to a clean vial and resuspend in 1 ml of complete medium. Seed cells into a T25 flask containing 5 ml of complete medium.
2. Incubate cells in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. Monitor cell growth and confluency.
3. Once cells reach 70-80% confluency, passage cells into a new T25 flask. Use 1 ml of medium from the old flask and 4 ml of fresh complete medium.
4. Repeat passage procedure as needed.
5. For long-term storage, harvest cells into a 15 ml centrifuge tube and resuspend in 10 ml of complete medium. Centrifuge at 300 x g for 5 minutes. Resuspend pellet in 1 ml of cryopreservation medium.
6. Aliquot 100 µl of cells into cryovials. Store in liquid nitrogen.
7. Thaw cryovials in a 37°C water bath. Do not vortex. Centrifuge at 300 x g for 5 minutes. Resuspend cells in 1 ml of complete medium.
8. Seed cells into a T25 flask containing 5 ml of complete medium. Incubate in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C.

**Incubation Atmosphere** 27°C, 0% CO<sub>2</sub>, humidified atmosphere.

**Shipping Conditions** Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately -78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

**Storage Conditions** For long-term preservation, place vials in vapor-phase liquid nitrogen at about -150 to -196 °C. Storage at -80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

**Genotype / Karyotype / HLA**

**Sterility** Genetically stable, mycoplasma free, and free of other contaminants. PCR confirmed negative for mycoplasma contamination.