



**Culture Medium** DMEM 4.5 g/l, Glucose 4 g/l, Sodium Pyruvate 3.7 g/l, NaHCO<sub>3</sub> 1.0 g/l, Penicillin (100 U/ml), Streptomycin (82 U/ml)

**Supplements** 10% FBS

**Dissociation Reagent** Trypsin

**Subculturing** Wash cells with PBS, trypsinize, and resuspend in fresh medium.

**Fluid renewal** 2-3 times per week

**Freeze medium** Serum-free medium (DMEM + 10% DMSO) + 10% FBS

- Thawing and Culturing Cells**
1. Thaw cells in a 37°C water bath.
  2. Dilute cells into fresh medium.
  3. Seed cells into a 25 cm<sup>2</sup> flask.
  4. Incubate at 37°C, 5% CO<sub>2</sub>.
  5. Monitor cell growth.
  6. Harvest cells at 70-80% confluency.
  7. Wash cells with PBS.
  8. Trypsinize cells.

**Incubation Atmosphere** 37°C, 5% CO<sub>2</sub>

**Flask Coating** None

