



OP9 | 305174

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**Culture Medium** MEM, w: 2.0 mM  $\beta$ -mercaptoethanol, w/o: penicillin, w/o: streptomycin, w: 1.0 mM nystatin, w: 2.2g/ml insulin-like growth factor 1

**Supplements** 20% FBS

**Dissociation Reagent** Trypsin-EDTA

**Subculturing** Cells are cultured in DMEM supplemented with 10% FBS. For subculturing, cells are trypsinized and resuspended in DMEM supplemented with 10% FBS. Cells are seeded into new flasks at a density of 1-2 x 10^5 cells per flask.

**Split ratio** 1:2 to 1:4

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

**Freeze medium** DMEM supplemented with 10% FBS + 10% DMSO

- Thawing and Culturing Cells**
1. Thaw the cells in a 37°C water bath.
  2. Dilute the cells into DMEM supplemented with 10% FBS.
  3. Seed the cells into a 25 cm<sup>2</sup> flask.
  4. Allow the cells to attach for 24 hours.
  5. Refresh the medium.
  6. Harvest the cells for cryopreservation.
  7. Seed the cells into a 25 cm<sup>2</sup> flask.
  8. Allow the cells to attach for 24 hours.

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

