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**Culture Medium** EMEM (MEM Eagle) 2 mM L-Glutamine - MEM Supplement 2.2 g/l NaHCO3 EBSS ( Gibco 820100a )

**Supplements** 10% FBS 1% Penicillin Streptomycin 1% Fungizone

**Dissociation Reagent** Trypsin

**Subculturing** Cells are harvested by trypsinization and centrifugation into PBS containing penicillin streptomycin fungizone

**Split ratio** 1:4 1:8

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

**Freeze medium** 10% FBS + 10% DMSO

- Thawing and Culturing Cells**
1. Thaw cells rapidly in a 37°C water bath.
  2. Centrifuge cells at 300 x g for 3 minutes.
  3. Wash cells three times with PBS containing penicillin streptomycin fungizone.
  4. Resuspend cells in 70% FBS.
  5. Seed cells into a T75 flask at 15 x 10^6 cells per flask.
  6. Seed cells into a 300 x cm^2 flask at 3 x 10^8 cells per flask.
  7. Seed cells into a 10 cm^2 flask at 1 x 10^7 cells per flask.
  8. Seed cells into a 25 cm^2 flask at 2.5 x 10^7 cells per flask.

**Incubation Atmosphere** 37°C 5% CO2

**Flask Coating** None

| 606465

**Freezing Procedure** [Redacted text] -78

**Shipping Conditions** [Redacted text] -78

**Storage Conditions** [Redacted text] -150 -196 [Redacted text]

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**Sterility** [Redacted text] (PCR) [Redacted text]  
[Redacted text]