



# HROG15 | 300937

**Culture Medium** DMEM:Ham's F12 (1:1), w: 3.1 g/L, w: 2.5 g/L L-Asparagine, w: 15 g/L HEPES, w: 0.5 g/L

**Supplements** FBS 10%

**Dissociation Reagent** Trypsin

**Subculturing** PBS, T25

**Seeding density**  $1 \times 10^4$  cells/cm<sup>2</sup>

**Fluid renewal** 3-5 days

**Freeze medium** 50% basal medium + 40% FBS + 10% DMSO, CM-1

- Thawing and Culturing Cells**
1. Thaw cryovial in 37°C water bath
  2. Transfer cells to cryovial and store at -150°C
  3. Incubate cells at 37°C
  4. Seed cells into T25 flask at 70% confluency
  5. Incubate cells for 15 days
  6. Harvest cells at 300 x g, 3 min
  7. Wash cells with PBS, 10 min
  8. Resuspend cells in fresh medium

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

