



# HROG36 | 300939

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

**Supplements** FBS 10%

**Dissociation Reagent** Trypsin

**Doubling time** 35 - 40 days

**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. Add to 10 ml of 70% FBS
  3. Incubate at 37°C for 15 min
  4. Wash cells in PBS
  5. Resuspend in 300 x g medium
  6. Seed into 3 wells
  7. Incubate at 37°C for 10 days
  8. Harvest cells

