



NCI-H661 | 305085

**Culture Medium** RPMI 1640, w: 2.0 mM  $\beta$ -mercaptoethanol, w: 2.0 g/L NaHCO<sub>3</sub> (Cytion 820700a)

**Supplements** 10% FBS

**Dissociation Reagent**

**Subculturing** Cells are harvested by trypsinization with Trypsin-EDTA (Cytion 820700a) and centrifuged at 300 x g for 5 min. Cells are resuspended in PBS (Cytion 820700a) and counted. Cells are seeded into flasks with 10% FBS (Cytion 820700a) and 10% DMSO (Cytion 820700a) in RPMI 1640 medium (Cytion 820700a).

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

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

**Freeze medium** RPMI 1640 medium (Cytion 820700a), 10% FBS (Cytion 820700a), 10% DMSO (Cytion 820700a)

- Thawing and Culturing Cells**
1. Thaw the cells in a water bath at 37°C. Centrifuge at 300 x g for 5 min. Resuspend in 10% FBS (Cytion 820700a) in RPMI 1640 medium (Cytion 820700a).
  2. Seed cells into flasks with 10% FBS (Cytion 820700a) in RPMI 1640 medium (Cytion 820700a) at 37°C. Incubate for 24-48 hours until cells reach 70% confluency.
  3. Harvest cells by trypsinization with Trypsin-EDTA (Cytion 820700a) and centrifuge at 300 x g for 5 min. Resuspend in PBS (Cytion 820700a) and count.
  4. Seed cells into flasks with 10% FBS (Cytion 820700a) in RPMI 1640 medium (Cytion 820700a) at 37°C. Incubate for 24-48 hours until cells reach 70% confluency.
  5. Harvest cells by trypsinization with Trypsin-EDTA (Cytion 820700a) and centrifuge at 300 x g for 5 min. Resuspend in PBS (Cytion 820700a) and count.
  6. Seed cells into flasks with 10% FBS (Cytion 820700a) in RPMI 1640 medium (Cytion 820700a) at 37°C. Incubate for 24-48 hours until cells reach 70% confluency.
  7. Harvest cells by trypsinization with Trypsin-EDTA (Cytion 820700a) and centrifuge at 300 x g for 5 min. Resuspend in PBS (Cytion 820700a) and count.
  8. Seed cells into flasks with 10% FBS (Cytion 820700a) in RPMI 1640 medium (Cytion 820700a) at 37°C. Incubate for 24-48 hours until cells reach 70% confluency.

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

**Flask Coating**

Product sheet

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**Freezing Procedure** [REDACTED] -78°C

**Shipping Conditions** [REDACTED] -78°C

**Storage Conditions** [REDACTED] -150 to 196

[REDACTED] / [REDACTED] / HLA

**Sterility** [REDACTED]  
[REDACTED]