



**HEK293T O-342 | 500305**

**Culture Medium** EMEM (MEM Eagle) 2 mM L-Glutamine-2-Mercaptoethanol 2.2 mM/100mM NaHCO3 5% EBSS ( Gibco 820100a 500ml)

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

**Dissociation Reagent** Trypsin

**Subculturing** Wash cells with PBS. Add 1ml of Trypsin to each well. Incubate for 5-10 minutes. Add 10ml of EBSS to stop the reaction. Pipette up the cells into a 15ml conical centrifuge tube. Centrifuge at 300g for 5 minutes. Remove the supernatant and resuspend the cell pellet in 1ml of EBSS.

**Split ratio** 1:4 1:6

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

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

- Thawing and Culturing Cells**
1. Thaw the vial in a 37°C water bath. Add the cells to 10ml of EBSS in a 15ml conical centrifuge tube. Centrifuge at 300g for 5 minutes. Remove the supernatant and resuspend the cell pellet in 1ml of EBSS.
  2. Seed the cells into a 24-well plate (100,000 cells/well) or a 96-well plate (20,000 cells/well). Incubate for 24 hours.
  3. Add 10ml of fresh medium to each well. Incubate for 37°C for 24 hours.
  4. Remove the medium and replace with fresh medium. Incubate for 70% confluency.
  5. Add 15ml of fresh medium to each well. Incubate for 8 days.
  6. Seed the cells into a 300cm<sup>2</sup> flask (3 x 10<sup>8</sup> cells/flask). Incubate for 24 hours.
  7. Add 10ml of fresh medium to each well. Incubate for 24 hours.
  8. Harvest the cells for downstream applications.

**Incubation Atmosphere** 37°C 5% CO2

**Flask Coating** None

