



**HEK293T RBE | 305019**

**Supplements**      10% FBS

**Dissociation Reagent**      Trypsin

**Subculturing**      Seed cells into 25 cm<sup>2</sup> flasks with 10% FBS medium. When cells reach 80-90% confluency, dissociate with Trypsin, wash with PBS, and seed into 75 cm<sup>2</sup> flasks with 10% FBS medium.

**Split ratio**      1:2 to 1:4

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

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

- Thawing and Culturing Cells**
1. Thaw vials in a 37°C water bath.
  2. Centrifuge at 300 x g for 3 minutes.
  3. Wash cells in PBS.
  4. Resuspend cells in 10% FBS medium.
  5. Seed cells into 25 cm<sup>2</sup> flasks.
  6. Incubate at 37°C with 5% CO<sub>2</sub>.
  7. Monitor cell growth.
  8. Harvest cells when they reach 80-90% confluency.

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

**Flask Coating**      None

**Freezing Procedure**      Seed cells into 25 cm<sup>2</sup> flasks with 10% FBS medium. When cells reach 80-90% confluency, dissociate with Trypsin, wash with PBS, and seed into 25 cm<sup>2</sup> flasks with 10% FBS medium.

