



**HEK293T HROG59 | 300880**

**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 and seed into 75 cm<sup>2</sup> flasks with 10% FBS medium. Split ratio 1:3.

**Freeze medium**      DMEM + 10% FBS + 10% DMSO, CM-1 (Cytion 800100)

**Thawing and Culturing Cells**

1. Thaw cells quickly in a 37°C water bath, then transfer to a 15 mL centrifuge tube and centrifuge at 300 x g for 3 minutes.
2. Remove the supernatant, wash cells with PBS, and resuspend in 10 mL of 10% FBS medium. Seed into a 75 cm<sup>2</sup> flask.
3. Incubate cells in a humidified 5% CO<sub>2</sub> incubator at 37°C until cells reach 80-90% confluency.
4. Dissociate cells with trypsin and centrifuge at 300 x g for 3 minutes. Resuspend in 10 mL of 10% FBS medium.
5. Seed cells into a 75 cm<sup>2</sup> flask with 10% FBS medium. Split ratio 1:3.
6. Incubate cells in a humidified 5% CO<sub>2</sub> incubator at 37°C until cells reach 80-90% confluency.
7. Dissociate cells with trypsin and centrifuge at 300 x g for 3 minutes. Resuspend in 10 mL of 10% FBS medium.
8. Seed cells into a 75 cm<sup>2</sup> flask with 10% FBS medium. Split ratio 1:3.

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

**Flask Coating**      None

**Freezing Procedure**      Seed cells into a 75 cm<sup>2</sup> flask with 10% FBS medium. When cells reach 80-90% confluency, dissociate with trypsin and seed into 15 mL centrifuge tubes with freeze medium. Freeze at -80°C.

**Shipping Conditions**      Store at -80°C

