



**HEK293T C918 | 305109**

**Supplements**      10% FBS

**Dissociation Reagent**      Trypsin

**Subculturing**      Seed cells into fresh medium containing 10% FBS. For passage 1, seed cells into T25 flasks. For passage 2-5, seed cells into 75 cm<sup>2</sup> flasks. For passage 6-10, seed cells into 175 cm<sup>2</sup> flasks. For passage 11-15, seed cells into 250 cm<sup>2</sup> flasks. For passage 16-20, seed cells into 500 cm<sup>2</sup> flasks. For passage 21-25, seed cells into 1000 cm<sup>2</sup> flasks. For passage 26-30, seed cells into 2000 cm<sup>2</sup> flasks. For passage 31-35, seed cells into 4000 cm<sup>2</sup> flasks. For passage 36-40, seed cells into 8000 cm<sup>2</sup> flasks. For passage 41-45, seed cells into 16000 cm<sup>2</sup> flasks. For passage 46-50, seed cells into 32000 cm<sup>2</sup> flasks. For passage 51-55, seed cells into 64000 cm<sup>2</sup> flasks. For passage 56-60, seed cells into 128000 cm<sup>2</sup> flasks. For passage 61-65, seed cells into 256000 cm<sup>2</sup> flasks. For passage 66-70, seed cells into 512000 cm<sup>2</sup> flasks. For passage 71-75, seed cells into 1024000 cm<sup>2</sup> flasks. For passage 76-80, seed cells into 2048000 cm<sup>2</sup> flasks. For passage 81-85, seed cells into 4096000 cm<sup>2</sup> flasks. For passage 86-90, seed cells into 8192000 cm<sup>2</sup> flasks. For passage 91-95, seed cells into 16384000 cm<sup>2</sup> flasks. For passage 96-100, seed cells into 32768000 cm<sup>2</sup> flasks.

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

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

**Thawing and Culturing Cells**

1. Thaw vials rapidly in a 37°C water bath. Transfer cells to a pre-warmed (37°C) DMEM medium containing 10% FBS.
2. Centrifuge cells at 300 x g for 3 minutes. Resuspend cells in 10 ml of pre-warmed (37°C) DMEM medium containing 10% FBS.
3. Seed cells into a pre-warmed (37°C) T25 flask containing 10 ml of pre-warmed (37°C) DMEM medium containing 10% FBS.
4. Seed cells into a pre-warmed (37°C) 75 cm<sup>2</sup> flask containing 25 ml of pre-warmed (37°C) DMEM medium containing 10% FBS.
5. Seed cells into a pre-warmed (37°C) 175 cm<sup>2</sup> flask containing 60 ml of pre-warmed (37°C) DMEM medium containing 10% FBS.
6. Seed cells into a pre-warmed (37°C) 250 cm<sup>2</sup> flask containing 80 ml of pre-warmed (37°C) DMEM medium containing 10% FBS.
7. Seed cells into a pre-warmed (37°C) 500 cm<sup>2</sup> flask containing 160 ml of pre-warmed (37°C) DMEM medium containing 10% FBS.
8. Seed cells into a pre-warmed (37°C) 1000 cm<sup>2</sup> flask containing 320 ml of pre-warmed (37°C) DMEM medium containing 10% FBS.

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

**Flask Coating**      None

**Freezing Procedure**      Seed cells into freezing medium containing 10% FBS + 10% DMSO. Seed cells into 15 ml x 8 ml or 15 ml x 15 ml flasks. Seed cells into 300 x g for 3 minutes. Resuspend cells in 10 ml of freezing medium. Seed cells into 1.8 ml cryovials. Freeze cells at -80°C.

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

