



Cell Culture Instruction for ProAdhere 293T Cells (Cat. No. LV592)

Freeze-Thaw Recovery

1. Pre-warm complete media in a 37°C waterbath.
2. Remove the cryopreserved vial from the liquid nitrogen storage tank.
3. Thaw the cells quickly by placing the lower half of the vial into the 37°C water bath and remove after 60 seconds. There should still be a few ice crystals left after thawing. It is important not to over-thaw the cryovials as the presence of DMSO is toxic to the cells.
4. Re-suspend the cells in the vial and transfer the cell suspension into a 15 mL sterile conical tube containing 5 mL complete media.
5. Centrifuge cells at 1500 rpm for 3 minutes to pellet.
6. Aspirate out the media, leaving cell pellet undisturbed.
7. Re-suspend pellet in fresh complete medium and plate in new culture vessel.
8. Incubate cultures at 37°C, 5% CO₂.

Propagation

The base medium for this cell line is Prigrow III medium available in ABM, Cat. No. [TM003](#). To make the complete growth medium, add the following components to the base medium: 10% fetal bovine serum ([TM999-500](#)) and 1% Penicillin/Streptomycin ([G255](#)).
Atmosphere: air, 95%; Carbon dioxide (CO₂), 5%.

Subculturing

1. Remove and discard culture medium.
2. Add enough Trypsin-EDTA solution ([TM050](#)) to cover the entire culture vessel and observe cells under an inverted microscope until cell layer is dispersed. The amount of Trypsin-EDTA varies depending on the size of the culture vessel (i.e. 2.0 ml is recommended for a 60 mm culture dish).

Note: Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

3. Add equal amount of complete media to stop the enzymatic reaction and collect all cells into a 15 mL sterile conical tube.
4. Centrifuge cells at 1500 rpm for 3 minutes to pellet.
5. Aspirate out trypsin, leaving pellet undisturbed.
6. Re-suspend pellet in fresh complete medium and plate in new culture vessel.
7. Incubate cultures at 37°C, 5% CO₂.

Preservation

- Freeze Medium: Complete growth medium with 10% DMSO.
- Storage Temperature: Liquid nitrogen vapour phase.