I. Notes

The Lenti-X 293T Cell Line (Cat. No. 632180) should be cultured immediately upon receipt, or as soon as possible thereafter. If culturing is significantly delayed after receipt, decreased cell viability may result. For HEK 293-based cell lines, we recommend using collagen-coated plates or flasks for the efficient culturing of frozen stocks. Vessels coated with compounds other than collagen may also provide suitable growth substrates (e.g. poly-L-lysine), but only collagen has been tested at Clontech. Once recovered, the cells may be cultured directly on tissue culture plastic. However, if adherence is poor, we recommend using only collagen-coated vessels.

II. Starting Lenti-X 293T Cell Line cultures from frozen stock:

To prevent osmotic shock and to maximize cell survival, perform the following:

1. Warm ~25 ml of complete culture medium in a 37°C water bath. See the Lenti-X 293T Cell Line Certificate of Analysis for medium composition.

   NOTE: Be sure to use the Tet System Approved Fetal Bovine Serum (Cat. Nos. 631101 & 631106) when using these cells with the Lenti-X HTX Packaging System (Cat. No. 631247).

2. Thaw vial of cells rapidly in a 37°C water bath with gentle agitation. Immediately upon thawing, remove from the water bath and wipe the outside of the vial with 70% ethanol. All of the operations from this point on should be carried out in a laminar flow tissue culture hood under strictly aseptic conditions. Unscrew the top of the vial slowly and, using a narrow pipet, transfer the contents of the vial to a 15 ml conical centrifuge tube containing 1 ml of prewarmed medium. Mix gently.

3. Slowly add an additional 4 ml of fresh, prewarmed medium to the tube and mix gently.

4. Add an additional 5 ml of prewarmed medium to the tube, mix gently. Centrifuge at 100 x g for 5 min, carefully aspirate the supernatant, and **gently** resuspend the cells in complete medium without selective antibiotics.

   NOTE: This method removes the cyropreservative and can be beneficial when resuspending in small volumes. However, be sure to treat the cells gently to prevent damaging fragile cell membranes.

5. Mix the cell suspension thoroughly and add to a suitable culture vessel. Gently rock or swirl the dish/flask to distribute the cells evenly over the growth surface and place it in a 37°C humidified incubator (5–10% CO_2 as appropriate) for 24 hr.

6. The next day, examine the cells under a microscope. If the cells are well-attached and confluent, they can be passaged for use. If the majority of cells are not well-attached, continue culturing for another 24 hr. Complete attachment of newly thawed cultures of HEK 293-based cell lines may require up to 48 hr.

7. For details on the use of these cells with the Lenti-X HTX Packaging System (Cat. No. 631247) and the Lenti-X Expression Systems, please refer to the Lenti-X Lentiviral Expression System User Manual.

III. Freezing the Lenti-X 293T Cell Line

Once the culture has been started and the cells are growing normally, you should prepare frozen aliquots to provide a renewable source of cells.

1. Trypsinize the desired number of flasks or plates.
2. Pool cell suspensions together, count cells, and calculate the total viable cell number.
3. Centrifuge cells at 100 x g for 5 min. Aspirate the supernatant.
4. Resuspend the pellet at a density of at least 1–2 x 10^6 viable cells/ml in freezing medium (Sigma-Aldrich Cat. No. C6164; or 70–90% FBS, 0–20% medium, and 10% DMSO).
5. Dispense 1 ml aliquots into sterile cryovials.
6. Freeze slowly (1°C per min). For this purpose, you can place the vials in Nalgene cryo-containers (Nalgene Cat. No. 5100) and freeze at –80°C overnight. Alternatively, place vials in a thick-walled styrofoam container at –20°C for 1–2 hr. Transfer to –80°C and freeze overnight. Remove vials from the cyro-containers or styrofoam containers the following day, and place in liquid nitrogen storage or ultralow-temperature freezer (–150°C) for storage.
7. Two or more weeks later, plate a vial of frozen cells to confirm viability.

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