I. Notes

The AAVpro 293T Cell Line (Cat. No. 632273) 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 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 by Clontech. Once recovered, the cells may be cultured directly on tissue culture-treated surfaces. However, if adherence is poor, we recommend using only collagen-coated vessels.

II. Starting AAVpro 293T Cell Line Cultures

To prevent osmotic shock and to maximize cell survival, follow the protocol below.

1. Warm ~25 ml of complete culture medium in a 37°C water bath. Refer to the AAVpro 293T Cell Line Certificate of Analysis for recommended medium composition.

2. Thaw one 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 and 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 damage to 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 incubation up to 48 hr.

7. For details on the use of these cells for helper virus-free preparation of AAV particles with the AAVpro Helper Free System (AAV2) (Cat. No. 6230), refer to the AAVpro Helper Free Systems User Manual available on www.clontech.com/manuals.

III. Freezing the AAVpro 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 cells from the desired number of flasks or plates.
2. Pool cell suspensions, 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 to 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). To do this, place the vials in a Nalgene freezing container (Thermo Scientific, Cat. No. 5100) and place 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 freezing container or Styrofoam container the following day, and place in liquid nitrogen storage or an 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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This document has been reviewed and approved by the Clontech Quality Assurance Department.