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The Adeno-X 293 Cell Line (Cat. No. 632271) has been developed and tested for use with the Adeno-X Expression Systems. For details on the use of these cells with Adeno-X Expression System 1 (Cat. No. 631513), refer to the Adeno-X Expression System 1 User Manual (PT3414-1). For details on the use of these cells with an Adeno-X Adenoviral System 3 (Cat. Nos. 632264, 632265, 632266, 632267, 632268, 632269 or 631180), refer to the Adeno-X Adenoviral System 3 User Manual (PT5177-1). For a complete listing of all available adenovirus products, visit **www.clontech.com**.

I. Starting Adeno-X 293 Cell Line cultures from frozen stock

IMPORTANT: Adeno-X 293 cells should be cultured immediately upon receipt, or as soon as possible thereafter. If culturing is significantly delayed after receipt, decreased cell viability may result. To prevent osmotic shock and to maximize cell survival, perform the following:

1. Thaw the 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 pipet, transfer the contents of the vial to a 15 ml conical centrifuge tube containing 1 ml of prewarmed medium (without selective antibiotics, e.g. G418). Mix gently.
2. 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 cryopreservative and can be beneficial when resuspending in small volumes. However, be sure to treat the cells gently to prevent damaging fragile cell membranes.)

3. 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.

   NOTE: 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-coated plates (e.g. BD BioCoat Cellware, Collagen Type I) have 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.

4. 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. Passage the cells 2−3 times before making frozen stocks or transfection/amplification.

   NOTE: Complete attachment of newly thawed cultures may require up to 48 hr.

5. Expand the culture as needed.

II. Freezing the Adeno-X 293 Cell Line

Once you have started growing the cell line, 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 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. Freezing medium can be purchased from Sigma Aldrich (Cat. Nos. C6164 & C6039); or freeze cells in 70–90% FBS, 0–20% medium (without selective antibiotics), and 10% DMSO.
5. Dispense 1 ml aliquots into sterile cryovials (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 cryo-containers or Styrofoam containers the following day, and place in liquid nitrogen storage or ultralow-temperature freezer (–150°C) for storage.
6. 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.