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I. Introduction

Clontech **Tet-Off® and Tet-On® Cell Lines** have been developed and functionally tested for use with the Tet Gene Expression Systems. Detailed instructions for their use are available in the Tet Systems User Manuals. For a complete listing of all available cell lines, visit **www.clontech.com**

II. Protocol

A. Starting Cultures from Frozen Stocks of Tet Cell Lines

IMPORTANT: Frozen cells should be cultured immediately upon receipt, or as soon as possible thereafter. If culturing after shipping is significantly delayed, decreased cell viability may result. To prevent osmotic shock and 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, 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 strict 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, 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. (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 hrs.

NOTES:

- 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 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.
- For Jurkat and other suspension cultures, suspend cells at a density of no less than 2×10^5 cells/ml.
- For NIH/3T3 cells, thaw and plate at a density of no more than 5×10^4 cells per ml.

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 hrs.

   NOTE: For HEK 293-based cell lines, complete attachment of newly thawed cultures may require up to 48 hrs.

5. Expand the culture as needed. Note: The appropriate selective antibiotic(s) may be added to the medium after 48–72 hr in culture.

B. Preparing Frozen Cultures of Tet Cell Lines

Once you have started growing a Tet System cell line—either a premade one from Clontech or one of your own cell lines—prepare frozen aliquots to ensure 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 x10^6 cells/ml in freezing medium. Freezing medium can be purchased from Sigma (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.
6. (Nalgene Cat. No. 5100) and freeze at –80°C overnight. Alternatively, place vials in a thick-walled styrofoam container at –20oC 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.
7. Two or more weeks later, plate a vial of frozen cells to confirm viability.

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