

293TT

293TT cells

Laboratory of Cellular Oncology

Overview

293TT cells [1] are useful for:

- Production of papillomavirus and polyomavirus-based reporter vectors ([pseudoviruses](#))
- Production of recombinant proteins in mammalian cells
- Propagation of torque teno viruses [2]
- Propagation of BK polyomavirus and JC polyomavirus primary isolates [3]

The National Cancer Institute's Developmental Therapeutics Program (DTP) distributes 293TT cells through its cell line [catalog](#).

Introduction

HEK-293 cells were originally generated by treating a human embryonic kidney homogenate with sheared adenovirus DNA [4, 5]. The nature of the parental cell type that gave rise to the resulting adenovirus-transformed cell line is uncertain. Based on gene expression profiling, a neuronal phenotype has been suggested [6]. However, this phenotype could theoretically have arisen from dysregulation of neuron restrictive silencer factor (gene symbol [REST](#)) or other changes occurring during the transformation of an originally non-neuronal cell lineage [7, 8]. HEK-293 cells are [severely aneuploid](#) and, like other tumor lines, their culture phenotype probably does not closely resemble the cell lineage of origin [9].

The 293T cell line was generated by introducing SV40 DNA into HEK-293 cells. 293T cells carry a stably integrated copy of the SV40 genome. Due to an unusual splicing bias, the 293T cell line expresses an unspliced SV40 early mRNA that encodes small t antigen [10]. 293T cells express very low amounts of Large T Antigen [11]. To generate 293TT cells, we stably transfected 293T cells with an SV40 Large T Antigen cDNA expression cassette (plasmid [pTIH](#)). The expression cassette contains a hygromycin resistance gene. 293TT cells should be cultured in 250 µg/ml hygromycin (we purchase stock solution from Roche) to promote maintenance of Large T Antigen expression.

293T cells lack several innate antiviral defense pathways [12-15]. At least one of these antiviral pathways is present in the original HEK-293 parent line. A commercial alternative to the 293TT line, called [293FT](#) (Life Technologies), is based on the HEK-293 parent. Although 293FT works well for pseudovirus production, in our hands 293TT outperforms 293FT cells in infectivity experiments. Theoretically, this may be due to innate antiviral factors present in 293FT but not 293TT.

293TT cells replicate plasmids with the SV40 origin of replication to very high copy number. This replication appears to occur with faster kinetics in 293TT cells than in 293FT cells, perhaps due to the 293TT line's expression of SV40 small t antigen. The 293FT line expresses only Large T Antigen (no small t).

The rapid over-replication of SV40 Ori+ plasmids in 293TT cells makes them useful for production of papillomavirus or polyomavirus reporter vectors ([pseudoviruses](#)). The 293TT line can also be used for production of recombinant proteins of interest. Recombinant protein production is best achieved by placing the gene of interest under control of human elongation factor 1 alpha (EF1) housekeeping promoter. Expression of genes under control of EF1 promoter appears to correlate with plasmid copy number. In contrast, expression driven by the more traditional CMV immediate early promoter appears less plasmid copy number dose-responsive. Presumably, some key transcription factor needed for CMV promoter is limiting in 293TT cells. The 293TT/EF1 system can result in extremely high expression of the recombinant protein (up to a few percent of total soluble protein in the cell). Examples of EF1 expression plasmids include the Gateway-adapted plasmids pGwf and pHf (see [plasmid maps](#)).

Thawing 293TT Cells

293TT cells are cultured in DMEM with 10% fetal calf serum (heat inactivated at 56°C for 30 minutes), 1% non-essential amino acids, and 1% Glutamax-I (DMEM-10). For the initial thaw of the 293TT cells, place the thawed suspension directly into a 225 cm² flask with 30 ml of DMEM with a total of 20% FCS. It is not necessary (or desirable) to spin the cells out of the freezing medium. Like other types of 293 cell lines, 293TT do not adhere tightly. It may take as many as three days for the cells to attach after thawing. If cells do not attach after two days, it may help to spin them out of the medium, wash once with calcium-free PBS, then pellet and resuspend for five minutes at 37°C in 1 ml of trypsin/EDTA. Resuspend the trypsin-treated cells in 30 ml of DMEM-10 and plate in a 225 cm² flask. The re-trypsinization helps dissociate large clumps and facilitates attachment of individual cells.

Passaging 293TT Cells

Split 293TT cells 1:5 to 1:20 when they reach 95% confluence. Detach cells by first gently rinsing the flask once with several ml of trypsin. It is important to add the trypsin along the top edge of the culture surface, allowing it to run toward the bottom of the flask in a sheet of fluid. Remove the first rinse of trypsin and sheet ~2 ml of fresh trypsin down the flask. Incubate the cells with trypsin in a humidified 37°C incubator for 5-10 minutes. 293TT cells adhere more tightly to one another than to standard culture plastic. It is therefore important to trypsinize the cells thoroughly. Insufficient trypsinization leads to lytic shredding of cell clumps during trituration (resuspension). Inactivate trypsin by adding 10 ml of DMEM-10. Resuspend the cells and transfer a portion of the cell suspension directly into a fresh flask. It is not necessary (or desirable) to spin the cells out of the residual trypsin, since the trypsin is functionally inactivated by the fetal calf serum in DMEM-10.

After the cells have fully recovered from thawing, DMEM-10 can be supplemented with 250 µg/ml hygromycin B (Roche) to promote maintenance of T antigen expression. Although 293TT cells can typically be passaged for several months without alteration of PsV production or titration characteristics, early passages should be frozen in aliquots for long-term storage.

293TT cells are sensitive to DMSO and freezing them improperly will result in failure to attach after thawing. It is important to chill all components needed to freeze down the cells prior to starting. This will minimize the length of time that the 293TT cells are exposed to DMSO. Begin with a T-225 flask of 293TT cells grown to ~80% confluence. Chill a rack with 8-10 labeled cryotubes to -20 °C. Chill a "Mr. Frosty" isopropanol bath to 4°C. Make 10 ml of 2x freeze medium (fetal calf serum + 18% DMSO) and chill on ice. Remove 5 ml of supernatant (conditioned medium) from the flask and save. Trypsinize the cells as described above (using 1-2 ml of trypsin), then resuspend in the reserved conditioned medium. Chill the suspension on ice. Mix the chilled cell suspension 1:1 with the chilled 2x freeze medium. Distribute 1.5 ml of suspension into each chilled cryotube. Place aliquots in Mr. Frosty and place at -80° C overnight, then transfer aliquots into liquid nitrogen for long-term storage.

Transfection

Use Invitrogen's Lipofectamine2000 essentially according to the package insert. Many other labs seem to have had good luck with Fugene 6 (Promega), but our lab is stuck with Lipofectamine2000 for historical reasons. Calcium phosphate transfection doesn't give good pseudovirion yields.

1. Preplate 7 million 293TT cells in 20 ml of DMEM-10 (no hygromycin or antibiotics) in a 75 cm² flask 16 hours prior to transfection. Cells should be ~40% confluent at the time of transfection. Higher density will result in reduced transfection efficiency. In general it appears that cells with a free edge attached to bare culture plastic are most efficiently transfected. Cells growing in the middle of islands (surrounded by other cells) seem not to be transfected well.
2. Mix 38 µg of DNA (for example, 19 µg each of p16sheLL and pYSEAP (see Notes 2 and 3 in main pseudovirus production [protocol](#)) with 2 ml of OptiMEM-I.
3. In a separate tube mix 85 µl of Lipofectamine 2000 with 2ml of OptiMEM-I.
4. Incubate the two mixtures separately at room temperature for 10 to 20 minutes, then combine and incubate for at least an additional 20 minutes.
5. Add the resulting lipid/DNA complexes directly to the preplated cell culture. It is not necessary to change medium prior to transfection (in fact, doing so appears to decrease transfection efficiency). From this point forward treat cells and all their products like an infectious agent (Note 4).
6. Incubate the cells with the lipid/DNA complexes overnight. The next morning, remove the complexes and add fresh DMEM-10 pre-warmed to 37°C. Add the fresh DMEM-10 to the upper surface of the flask to avoid dislodging cells. Previous versions of this protocol specified incubating the cells with the lipid/DNA complexes for 4-6 hours. Although the overnight incubation causes noticeable cytotoxicity, we have found that the overall titer yields are nevertheless somewhat better from overnight-transfected cultures, particularly if the cell density is higher than 50%.
7. After the media change, return flask to the incubator for ~30 hours (i.e., a total of 48 hours after initial addition of the lipid/DNA complexes). It is not necessary to split the culture.

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