

293T/GFP-Puro Cell Line

CATALOG NUMBER: AKR-202

STORAGE: Liquid nitrogen

Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80°C until first culture. Store subsequent cultured cells long term in liquid nitrogen.

QUANTITY & CONCENTRATION: 1 mL, 1×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO

Background

The 293 cell line is a permanent line established from primary embryonic human kidney transformed with human adenovirus type 5 DNA. The genes encoded by the E1 region of adenovirus (E1a and E1b) are expressed in these cells and participate in transactivation of viral promoters, allowing these cells to produce high levels of protein. E1 also complements the E1-deletion in recombinant adenoviral vectors, allowing viral replication. Our 293T/GFP-Puro cell line stably expresses the SV40 large T antigen, GFP-Puro fusion gene, and Neomycin resistance gene.

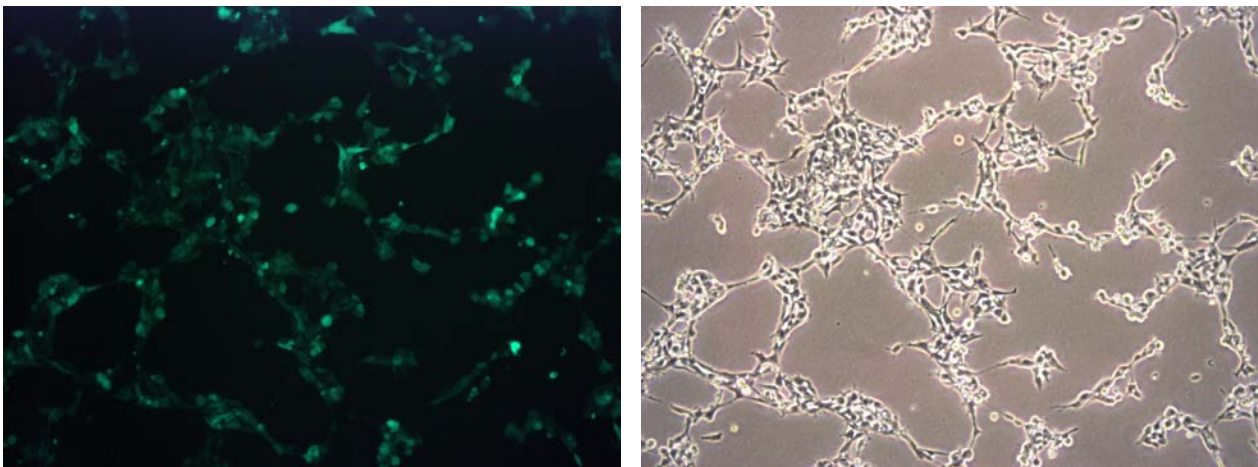


Figure 1. 293T/GFP-Puro Cell Line. Left: GFP Fluorescence; Right: Phase Contrast.

Quality Control

This cryovial contains at least 1.0×10^6 293T/GFP-Puro cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The 293T/GFP-Puro cells are tested free of microbial contamination.

Medium

1. Culture Medium: D-MEM (high glucose), 10% fetal bovine serum (FBS), 0.1 mM MEM Non-Essential Amino Acids (NEAA), 2 mM L-glutamine, 1% Pen-Strep, (optional) 2 μ g/mL Puromycin.
2. Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO.

Methods

Establishing 293T/GFP-Puro Cultures from Frozen Cells

1. Place 10 mL of complete DMEM growth medium in a 50-mL conical tube. Thaw the frozen cryovial of cells within 1–2 minutes by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
2. Transfer the thawed cell suspension to the conical tube containing 10 ml of growth medium.
3. Collect the cells by centrifugation at 1000 rpm for 5 minutes at room temperature. Remove the growth medium by aspiration.
4. Resuspend the cells in the conical tube in 15 mL of fresh growth medium by gently pipetting up and down.
5. Transfer the 15 mL of cell suspension to a T-75 tissue culture flask. Place the cells in a 37°C incubator at 5% CO₂.
6. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

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