

## 293/GFP Cell Line

**CATALOG NUMBER:** AKR-200

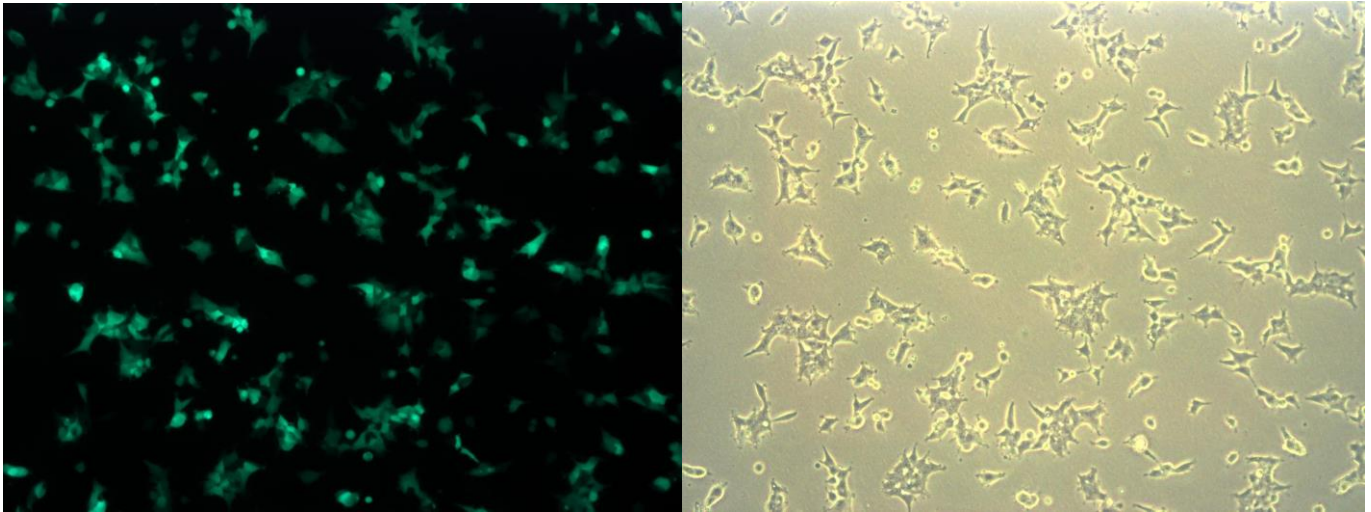
**STORAGE:** Liquid nitrogen

*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,  $1 \times 10^6$  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 293/GFP cell line stably expresses GFP and blasticidin-resistant genes. Both GFP and blasticidin-resistant genes are introduced into parental 293 cells using lentivirus.



**Figure 1.** 293/GFP Cell Line. Left: GFP Fluorescence; Right: Phase Contrast.

### **Quality Control**

This cryovial contains at least  $1.0 \times 10^6$  293/GFP cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The 293/GFP 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) 10  $\mu$ g/mL Blasticidin.
2. Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO.

## **Methods**

### **Establishing 293/GFP 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% CO<sub>2</sub>.
6. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

### **Recent Product Citations**

1. Lainšček, D. et al. (2022). Coiled-coil heterodimer-based recruitment of an exonuclease to CRISPR/Cas for enhanced gene editing. *Nat Commun.* **13**(1):3604. doi: 10.1038/s41467-022-31386-1.
2. Suzuki, Y. et al. (2020). Lipid nanoparticles loaded with ribonucleoprotein-oligonucleotide complexes synthesized using a microfluidic device exhibit robust genome editing and hepatitis B virus inhibition. *J Control Release.* doi: 10.1016/j.jconrel.2020.12.013.
3. Bondarenko, G. et al. (2020). Semiquantitative Methods for GFP Immunohistochemistry and In Situ Hybridization to Evaluate AAV Transduction of Mouse Retinal Cells Following Subretinal Injection. *Toxicol Pathol.* doi: 10.1177/0192623320964804.
4. Lainšček, D. et al. (2018). Delivery of an artificial transcription regulator dCas9-VPR by extracellular vesicles for therapeutic gene activation. *ACS Synth Biol.* doi: 10.1021/acssynbio.8b00192.
5. De Los Reyes-Berbel, E. et al. (2018). PEI-NIR Heptamethine Cyanine Nanotheranostics for Tumor Targeted Gene Delivery. *Bioconjug Chem.* **29**(8):2561-2575. doi: 10.1021/acs.bioconjchem.8b00262.
6. Irvine, S. A. et al. (2015). Printing cell-laden gelatin constructs by free-form fabrication and enzymatic protein crosslinking. *Biomed Microdevices.* **17**:1-8.

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