# NIH3T3/GFP Cell Line

**CATALOG NUMBER:** AKR-214

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 x 10<sup>6</sup> cells/mL in 70% DMEM, 20% FBS, 10% DMSO

## **Background**

NIH 3T3 cells are established from a NIH Swiss mouse embryo. These cells are highly contact inhibited and are sensitive to sarcoma virus focus formation and leukaemia virus propagation. Cells have now lost their contact inhibition. The established NIH/3T3 line was subjected to more than 5 serial cycles of subcloning in order to develop a subclone with morphologic characteristics best suited for transformation assays. The "3T3" designation refers to the abbreviation of "3-day transfer, inoculum 3 x 10<sup>5</sup> cells." The NIH3T3 cell line is one of the most commonly used fibroblast cell lines. Our NIH3T3/GFP cell line stably expresses GFP and blasticidin-resistant genes. Both GFP and blasticidin-resistant genes are introduced into parental NIH3T3 cells using lentivirus.

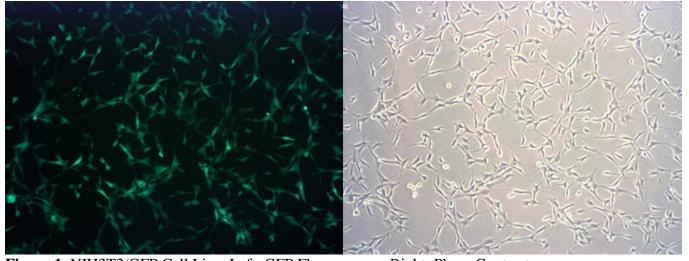


Figure 1. NIH3T3/GFP Cell Line. Left: GFP Fluorescence; Right: Phase Contrast.

#### **Quality Control**

This cryovial contains at least  $1.0 \times 10^6$  NIH3T3/GFP cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The NIH3T3/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 μg/mL Blasticidin.
- 2. Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO.

#### Methods

#### **Establishing NIH3T3/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% CO2.
- 6. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

## **Recent Product Citations**

- 1. Weems, A.C. et al. (2017). Shape memory polyurethanes with oxidation-induced degradation: in vivo and in vitro correlations for endovascular material applications. *Acta Biomater*. doi:10.1016/j.actbio.2017.06.030.
- 2. Bouchlaka, M.N. et al. (2017). Human Mesenchymal Stem Cell-Educated Macrophages Are a Distinct High IL-6-Producing Subset that Confer Protection in Graft-versus-Host-Disease and Radiation Injury Models. *Biol Blood Marrow Transplant*. pii: S1083-8791(17)30306-3. doi: 10.1016/j.bbmt.2017.02.018.
- 3. Pearson, R. A. et al. (2016). Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nat Commun*. doi:10.1038/ncomms13029.
- 4. Nash, L. D. et al. (2016). Cold plasma reticulation of shape memory embolic tissue scaffolds. *Macromol Rapid Commun*. doi:10.1002/marc.201600268.
- 5. Castleberry, S. A. et al. (2016). Nanolayered siRNA delivery platforms for local silencing of CTGF reduce cutaneous scar contraction in third-degree burns. *Biomaterials*. doi:10.1016/j.biomaterials.2016.04.007.
- 6. Castleberry, S. A. et al. (2015). Self-assembled wound dressings silence MMP-9 and improve diabetic wound healing in vivo. *Adv Mater*. doi:10.1002/adma.201503565.
- 7. Peak, C. W. et al. (2015). Elastomeric cell-laden nanocomposite microfibers for engineering complex tissues. Cell Mol Bioeng. doi:10.1007/s12195-015-0406-7.
- 8. Tassoni, A. et al. (2015). Molecular mechanisms mediating retinal reactive gliosis following bone marrow mesenchymal stem cell transplantation. *Stem Cells*. doi: 10.1002/stem.2095.
- 9. Scott, C. M. et al. (2015). 3D cell entrapment as a function of the weight percent of peptide-amphiphile hydrogels. *Langmuir*. doi:10.1021/acs.langmuir.5b00196.



10. Jo, W. et al. (2014). Microfluidic fabrication of cell-derived nanovesicles as endogenous RNA carriers. *Lab Chip.* **14**:1261-1269.

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