

NIH3T3/Cas9 Cell Line

CATALOG NUMBER: AKR-5104

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 10⁶ 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 NIH3T3 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 10⁵ cells.” The NIH3T3 cell line is one of the most commonly used fibroblast cell lines. Our NIH3T3/Cas9 cell line stably expresses Cas9 and puromycin-resistant genes. Both Cas9 and puromycin-resistant genes were introduced into parental NIH3T3 cells using retrovirus.

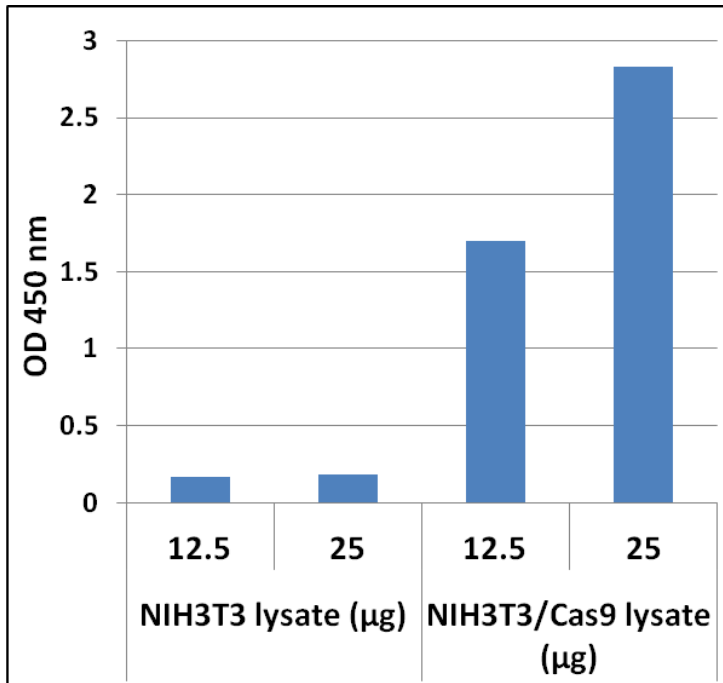


Figure 1. NIH3T3/Cas9 Cell Line Expression of Cas9 Protein. NIH3T3 cells or NIH3T3/Cas9 cells were lysed with RIPA buffer and tested for Cas9 protein levels using Cell Biolab’s Cas9 ELISA Kit (Cat# PRB-5079).

Quality Control

This cryovial contains at least 1.0×10^6 NIH3T3/Cas9 cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The NIH3T3/Cas9 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) 1-2 $\mu\text{g/mL}$ Puromycin.
2. Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO.

Methods

Establishing NIH3T3/Cas9 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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