

Data Sheet

NP69 Nasopharyngeal Epithelial Cell Line

Immortalized Cell Line

SCC197**Pack Size: $\geq 1 \times 10^6$ viable cells/vial****Store in liquid nitrogen.****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for Human or Animal Consumption.**

Background

Nasopharyngeal carcinoma (NPC), the most common cancer arising in the nasopharyngeal region, arises from epithelial cells and is especially prevalent in areas of southeast Asia and as a childhood cancer in some African populations.¹ NPC ontology and tumorigenesis is closely associated with viral factors, especially Epstein-Barr virus (EBV) and human papillomavirus (HPV).¹ The NP69SV40T immortalized human nasopharyngeal epithelial cell line retains many of the characteristics of normal nasopharyngeal cells, including keratin profiles and response to TGF-beta, while possessing many of the genetic signatures observed in NPC.² NP69 cells are non-tumorigenic and demonstrate anchorage-dependent growth.² The NP69 cell line is particularly useful for studies of the mechanisms of viral-associated tumorigenesis.

Source

NP69 cells were derived from passage 1 cultures of biopsy-isolated primary nasopharyngeal epithelial cells transfected with the PX8 plasmid containing the simian virus 40 large T antigen.¹

Short Tandem Repeat

| | |
|-----------------|------------------|
| D3S1358: 17 | D16S539: 11, 12 |
| TH01: 7 | CSF1PO: 12, 13 |
| D21S11: 31 | Penta D: 8 |
| D18S51: 13 | vWA: 16, 19 |
| Penta E: 11, 16 | D8S1179: 15, 16 |
| D5S818: 11 | TPOX: 11 |
| D13S317: 10, 12 | FGA: 21, 22 |
| D7S820: 11 | Amelogenin: X, Y |

Cancer cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- Each vial contains $\geq 1 \times 10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from rat, mouse, Chinese hamster, Golden Syrian hamster, and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.
- Each lot of cells are genotyped by STR analysis to verify the unique identity of the cell line.

Storage and Handling

NP69 Nasopharyngeal Epithelial cell line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

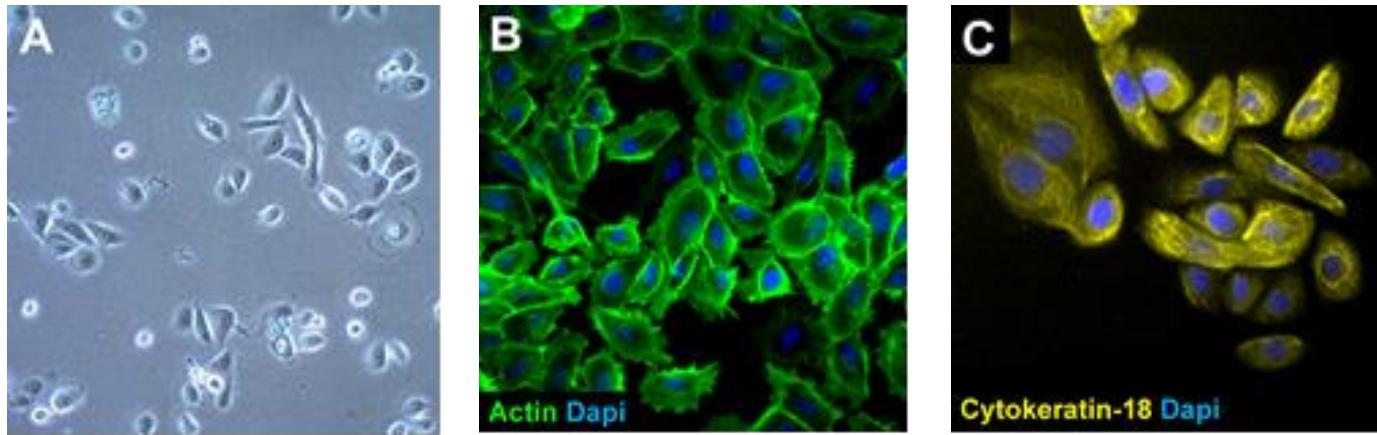


Figure 1: **A.** NP69 cells one day after thawing in a T75 flask. **B.** Cells express actin (P5282) and **C.** cytokeratin-18 (MAB3234).

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.
2. NP69 Expansion Medium: Cells are thawed and expanded in Keratinocyte Serum-Free Growth Medium (Promocell, C-20211) supplemented with 0.2 ng/mL EGF (GF144), 25 µg/mL bovine pituitary extract (02-103) and 2% FBS (ES-009-B).
3. Remove the vial of frozen NP69SV40T cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

IMPORTANT: Do not vortex the cells.

4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
5. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
6. Using a 10 mL pipette, slowly add dropwise 9 mL of NP69 Expansion Medium (Step 1 above) to the 15 mL conical tube.

IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.

7. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.
8. **IMPORTANT:** Do not vortex the cells.
9. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
10. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
11. Resuspend the cells in 15 mL of NP69SV40T Expansion Medium.
12. Transfer the cell mixture to a T75 tissue culture flask.
13. Incubate the cells at 37 °C in a humidified incubator with 5% CO₂.

Subculturing Cells

Do not grow NP69SV40T cells to confluence as this will make it difficult to detach the cells and may cause cell clumping. Passage when the cells are at ~85% confluence will make it easier to detach the cells and avoid cell clumping.

1. Do not allow the cells to grow to confluence. NP69SV40T cells should be passaged at ~80-90% confluence at a split ratio of 1:6.
2. Carefully remove the medium from the T75 tissue culture flask containing the NP69SV40T cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase® and incubate in a 37 °C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of NP69SV40T Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of NP69SV40T Expansion Medium to the conical tube and resuspend the cells thoroughly.

IMPORTANT: Do not vortex the cells.

11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

NP69SV40T Nasopharyngeal Epithelial Cell Line may be frozen in the Expansion Medium plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

Reference

1. Lancet 2016; 387(10022): 1012-1024.
2. Biochim Biophys Acta 2002; 1590(1-3): 150-158.

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