NP69 Nasopharyngeal Epithelial Cell Line

Immortalized Cell Line

Cat. # SCC197

Pack size: ≥1x10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS

Data Sheet

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 (STR) Profile

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.

Storage & 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.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ 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 is genotyped by STR analysis to verify the unique identity of the cell line.

Representative Data

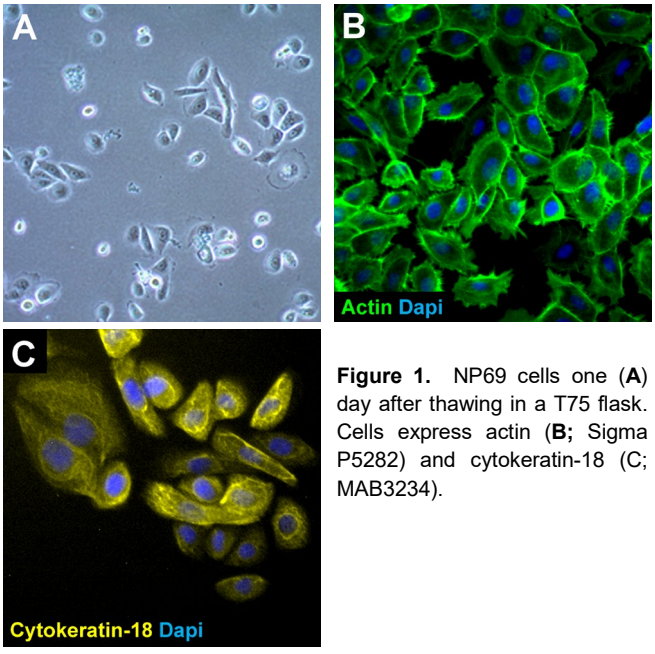

Figure 1. NP69 cells one (**A**) day after thawing in a T75 flask. Cells express actin (**B;** Sigma P5282) and cytokeratin-18 (C; MAB3234).

References

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

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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.

   NP69 Expansion Medium: Cells are thawed and expanded in Keratinocyte Serum-Free Growth Medium (Sigma Cat. No. 131-500A) supplemented with 0.2 ng/mL EGF (Cat. No. GF144), 25 μg/mL bovine pituitary extract (Cat. No. 02-103) and 2% FBS (Cat. No. ES-009-B).
2. 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.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. 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.
5. 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.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of NP69SV40T Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.

Subculturing Cells

Note: 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 confluency. 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.
3. Apply 5-7 mL of Accutase and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 5-7 mL of NP69SV40T Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of NP69SV40T Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. 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.

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