SF7761 Human DIPG H3.3-K27M Cell Line

Cancer Cell Line

Cat. # SCC126

Pack size: ≥ 200 neurospheres/vial

Store in liquid nitrogen

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Certificate of Analysis

Background

Diffuse intrinsic pontine gliomas (DIPG) are highly aggressive and difficult to treat tumors arising in the ventral pons of the brain stem. Despite therapeutic advances, DIPG is incurable and most patients, primarily children, die within 2 years of diagnosis. DIPD is one of the leading causes of death in children with brain tumors[1].

A somatic mutation of histone H3.3 resulting in a lysine 27 to methionine substitution (H3.3K27M) occurs in 60% of DIPG[2]. In H3.3K27M DIPG patient samples, levels of H3K27 dimethylation (H3K27me2) and trimethylation (H3K27me3) are reduced globally. Expression of H3.3K27M was also shown to be associated with increased levels of H3K27 acetylation (H3K27ac) and recruitment of bromodomain proteins at sites of active transcription[4]. These epigenetic changes are thought to be important factors driving DIPG oncogenesis[2,3]

SF7761 is a human glioma cell line derived by surgical biopsy from a young female H3.3K27M DIPG patient[5]. The tumor cells were immortalized with hTERT (human telomerase ribonucleoprotein reverse transcriptase) using retroviral transduction. SF7761 cells are tumorigenic in athymic rodents with the tumor cells recapitulating the infiltrative growth of human brainstem gliomas[5, 6].

Short Tandem Repeat (STR) Profile

D3S1358: 15, 17	D16S539: 11
TH01: 7, 9.3	CSF1PO: 13
D21S11: 28, 31.2	Penta D: 13
D18S51: 19, 22	vWA: 14, 18
Penta E: 14, 17	D8S1179: 11, 15
D5S818: 11, 12	TPOX: 11, 12
D13S317: 8, 9	FGA: 20
D7S820: 10, 11	Amelogenin: X

Tumor 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 and Handling

SF7761 Human DIPG H3.3-K27M 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 ≥ 200 viable neurospheres.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, C, Herpesvirus type 6, 7, 8 and HIV-1 & 2 viruses by PCR.
- 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

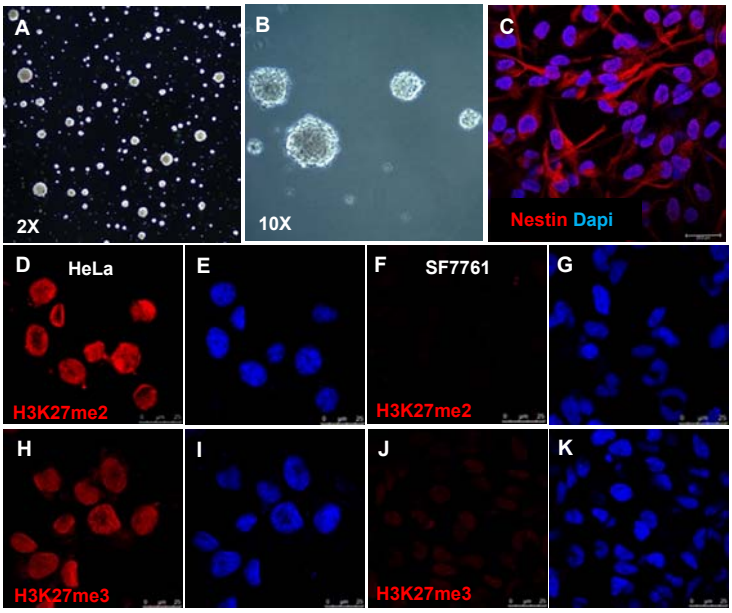

Figure 1: Day 1 after thaw, 2X and 10X magnification, respectively (**A, B**). SF7761 cells express Nestin (**C**). H3K27me2 and H3K27me3 signals are reduced in SF7761 (**F, J**) respectively compared with the HeLa cell positive control (**D, H**). Cells were plated on poly-D-lysine and laminin coated cover glass bottom chamber slides. Anti-Nestin, clone 10C2, Cy3 conjugate (Cat# MAB5326C3), anti-H3K27me2 (Cell Signaling Cat# 9728), anti-H3K27me3 (Cat# ABE44) and Alexa Fluor 647 Goat anti-Rabbit IgG (Cat# AP187SA6) were used.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Rev. 2.0/2017-06-02/SCC126/AN

Protocols

Note: SF7761 are grown as neurospheres in suspension culture. Media replacement should be performed twice a week with growth medium made fresh each week. Neurospheres are to be passaged using mechanical trituration when they reach diameters of 200-500 μm or through enzymatic dissociation.

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.

   Cells are thawed and expanded in ReNcell NSC Maintenance Medium (Cat. No. SCM005), containing 20 ng/mL EGF (Cat. No. GF001), 20 ng/mL FGF-2 (Cat. No. GF003) and 1X Penicillin-Streptomycin Solution (EMD Millipore Cat. No. TMS-AB2-C).

   Note: *Complete Supplemented ReNcell NSC Medium is stable for only 6 days at 2-8C. It is thus important to make fresh media supplement with growth factors once a week.*

2. Remove the vial of frozen SF7761 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 SF7761 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 10-15 mL of SF7761 Expansion Medium. Transfer the cell mixture to a T75 tissue culture flask. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.
10. Exchange with 10 – 15 mL fresh SF7761 Expansion Medium every two to three days thereafter.
13. For media changes, transfer cell suspension to a 50 mL conical tube and centrifuge at 300 x g for 2-3 minutes to pellet cells. Aspirate. Resuspend cell pellet in 10 – 15 mL fresh SF7761 Expansion medium. Incubate cells in 37°C humidified incubator with 5% CO_2.

Subculturing Cells

Mechanical Dissociation Method:

1. Check SF7761 neurosphere culture daily.
2. When the majority of neurospheres reach 200 – 500 μm, pellet the cells and resuspend in 1 mL SF7761 Expansion medium.
3. Using a 1 mL pipetteman and pipet tip, gently triturate the cell suspension several times to break the large neurospheres.
4. Replate triturated cell suspension into fresh SF7761 Expansion Medium and transfer to a fresh flask. Depending on the density of the neurospheres, cells may be replated in 15 – 30 mL fresh medium for a T75 flask or 45 – 75 mL fresh media for a T225 flask.
5. Fresh media replacement should be performed twice a week with the growth medium made fresh each week.

Enzymatic Dissociation Method:

1. Collect neurospheres to a 50 mL conical tube. Centrifuge at 300 x g for 2-3 minutes to pellet the cells. Aspirate.
2. Add 7 mL of TrypLE Express (LIFE Cat No. 12604-039) and 70 μL DNAse I (Worthington Cat. No. LS002007) to the cell pellet. Triturate a few times with a 10 mL pipette.
3. Gently rotate the conical tube in a 37°C nutator (pre-warmed) for 10 minutes. Make sure that the lid of the tube is closed properly to avoid leakage.
4. Triturate the cells with a 10 mL pipette to dissociate the cells.
5. Add 22 mL of pre-warmed HBSS to the cell mixture. Mix the solution. Centrifuge at 300 x g for 7 minutes. Aspirate the supernatant.
6. Resuspend the cells in 2 mL fresh medium and do a cell count. Replate cell suspension to a fresh flask with an appropriate volume of fresh media.

Cryopreservation of Cells

SF7761 Human Glioma Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Schroeder KM et al. (2014) *Pediatr. Res*. 75(1-2): 205–209.
2. Lewis PW et al. (2013) *Science* 340(6134): 857–861.
3. Chan KM et al. (2013) *Genes & Development* 27(9): 985-990.
4. Piunti A et al. (2017) *Nat. Med.* 23(4): 493-500.
5. Hashizume R et al. (2012) *Neuro Oncol.* 110(3): 305-13.
6. Hashizume R et al. (2014) *Nat Med* 20(12): 1394-6.

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