

Data Sheet

BCH869 Human H3K27M Glioma Cell Line

Tumor Cell Line

SCC218**Pack Size: $\geq \times 10^6$ viable****Store in liquid nitrogen.****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for Human or Animal Consumption.**

Background

Pediatric high-grade glioma (pHGG) and diffuse intrinsic pontine glioma (DIPG) are among the most aggressive and recalcitrant cancers, with extremely low survival rates. Diffuse midline gliomas (DMG) with histone H3 lysine 27-to-methionine mutations (H3K27M glioma) represent a highly aggressive subtype of glioma, which predominantly arise in children and young adults. The overall prognosis of H3K27M glioma is poor, displaying median survival rates of approximately 9 to 11 months.^{1,2} Aberrant activation of the PI3K pathway via *PTEN* silencing is a contributor to tumorigenesis in most pHG and DIP gliomas.

The BCH869 H3K27M glioma cell line is a representative model for H3K27M diffuse glioma. BCH869 cells grow as neurospheres and carry mutations in oncogenes *ACVR1*, *PIK3CA*, and *PPM1D* in addition to H3K27M mutation.³ BCH869 cells express EGFR, a common glioma marker, and have been validated via staining for the H3.3 K27M oncohistone mutant. BCH869 cell line has been used in the investigation of radiosensitization factors and tumorigenesis pathways and is a highly clinically relevant model for this especially serious form of pediatric glioma.

Source

BCH869 cell line was derived from Pons tissue of a 7-year-old female patient.⁴

Short Tandem Repeat

D3S1358:	16, 18	D8S1179:	11, 12	D13S317:	12, 14	CSF1PO:	10
D7S820:	10, 11	D21S11:	30, 30.2	D16S539:	11, 13	Amel:	X
vWA:	16, 17	D18S51:	16, 19	TH01:	6, 7	Penta D:	12, 13
FGA:	23, 24	D5S818:	9, 11	TPOX:	8	Penta E:	7, 12

Cancer cell lines are inherently genetically unstable. 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 mouse, rat, 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.

Storage and Handling

BCH869 Human H3K27M glioma cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Representative Data

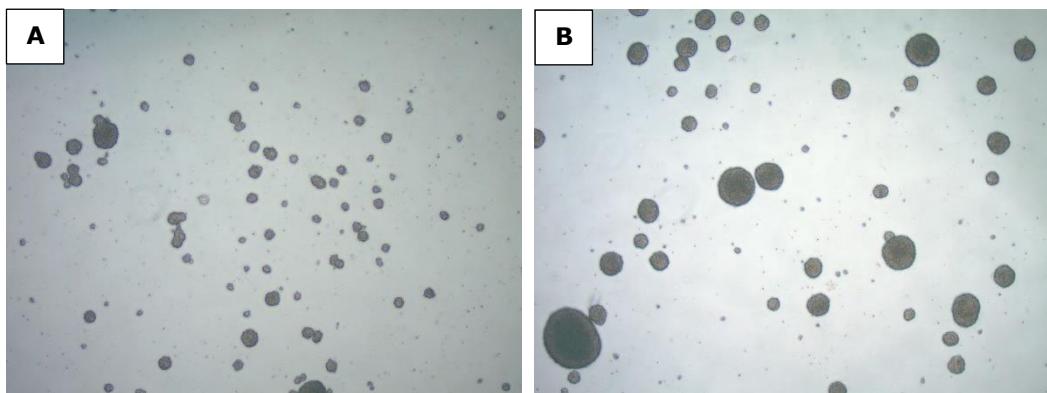


Figure 1. Bright-field images of BCH869 Human H3K27M glioma cells in culture, three (A) and seven (B) days after thaw in an ultra-low attachment T75 flask.

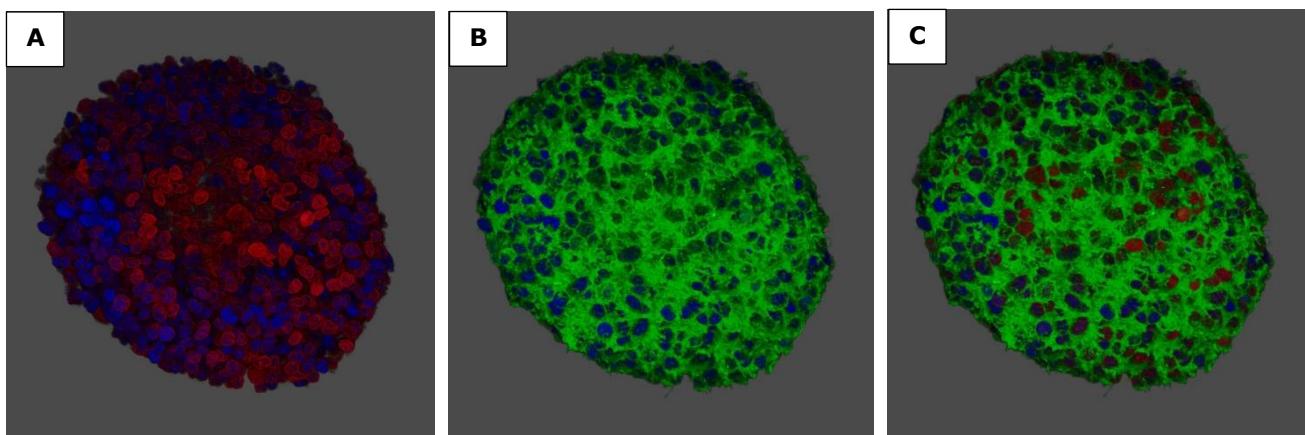


Figure 2. 3D images of BCH869 Human H3K27M glioma cells. Cells express phosphatidylinositol 3-kinase (PIK3CA) (A, red) and EGFR (B, green). Merged images (C).

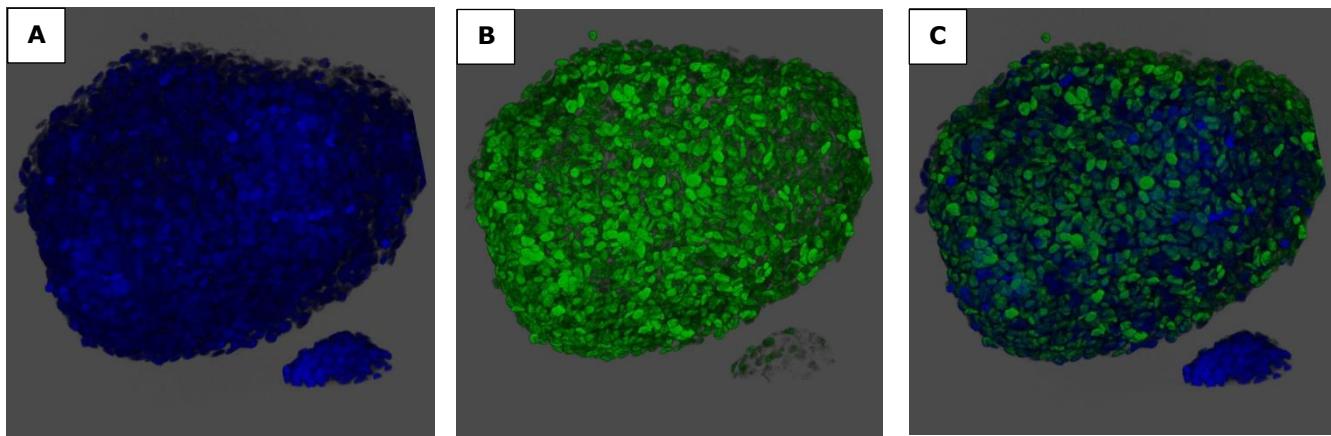


Figure 3. 3D images of BCH869 Human H3K27M glioma cells. Cells express H3K27M (B, green). Nuclei were counterstained with Dapi (A, blue). Merge images (C).

Protocols

BCH869 Human H3K27M glioma cells grow as neurospheres and should be cultured on ultra-low attachment T75 flasks (3814, Corning®). The cultures should be fed twice a week with growth medium that is made fresh each week. Neurospheres should be passaged every 7–8 days by dissociating into single cells by mechanical dissociation (tituration). Do not use trypsin to dissociate the cells as this will result in viability loss and should thus be avoided.

1. Prepare BCH869 Expansion Medium (100 mL).

Component	Quantity	Final Conc.	Supplier	Catalogue No
Neurobasal-A Medium (1X)	46.6 mL		ThermoFisher	10888-022
DMEM/F12 Medium	46.6 mL		Sigma	DF-041-B
HEPES Buffer, 1M Solution	1 mL	10 mM	Sigma	TMS-003-C
MEM Sodium Pyruvate Solution (100 mM; 100X)	1 mL	1 mM	Sigma	TMS-005-C
MEM, Non-Essential Amino Acids (100X)	1 mL	1X	Sigma	TMS-001-C
Ala-Gln (100X)	1 mL	1X	Sigma	G8541-100mL
B27 Supplement minus Vitamin A (50X)	2 mL	1X	ThermoFisher	12587-010
EGF, 20 µg/mL stock (1000X)	100 µL	20 ng/mL	Sigma	01-107
FGF-2, 20 µg/mL stock (1000X)	100 µL	20 ng/mL	Sigma	GF003AF-100UG
Platelet Derived Growth Factor-AA (PDGF-AA, 20 µg/mL stock; 2000X)	50 µL	10 ng/mL	Sigma	GF142
Platelet Derived Growth Factor-BB (PDGF-BB, 20 µg/mL stock; 2000X)	50 µL	10 ng/mL	Sigma	GF149
Heparin (10000 U/mL stock)	10 µL	1 U/mL	Sigma	375095-100KU
Antibiotic-Antimycotic Solution (100X); optional	1 mL	1X	Sigma	A5955-100ML

2. Remove the vial of frozen BCH869 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 BCH869 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 BCH869 Expansion Medium.
10. Transfer the cell mixture to an ultra-low attachment T75 flask (3814, Corning®).
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO₂.
12. Because of the instability of the media supplements and growth factors, BCH869 neurosphere culture should be fed every 2–3 days.
13. For media exchanges: Transfer neurosphere suspension to a 15 mL or 50 mL conical tube. Pellet the neurospheres in a centrifuge at 1200 rpm (220 x g) for 5 minutes. Aspirate, leaving behind a little media. Using a 5 mL pipette, resuspend the cell pellet in 15 mL fresh medium. Pipette up and down 5X with a serological pipette. Plate cells back to ultra-low attachment T75 flask (3814, Corning®).
14. Passaging BCH869 neurospheres as single cell suspension: BCH869 neurospheres should be passaged every 7–8 days. Cells are dissociated to single cell suspension by mechanical titration. Immediately after passage, refresh media on days 3 or 4. Thereafter, exchange with fresh media every 2 to 3 days until ready to passage.

Note: Do not use Trypsin to dissociate the cells. BCH869 neurospheres grow much slower than regular monolayer cell lines. Therefore, you will need to prepare at least double the number of flasks to achieve the same number of cells as monolayer culture.

15. Collect neurospheres and centrifuge at 1200 rpm (220 x g) for 5 minutes. Aspirate, leaving behind a little media.
16. Using a p1000 pipettman (Note: Do not use a serological pipette), resuspend the cell pellet in 600 µL expansion medium. Pipette up and down 50 times with the pipette tip touching the bottom of the conical tube to obtain a single cell suspension. Avoid the generation of air bubbles.
17. Live cells count with Tryphan Blue: Take 5 µL of single cell suspension and resuspend in 45 µL culture medium. Add 50 µL Tryphan Blue (Final 20X dilution). Count number of live cells using hemacytometer.
18. Plate approximately 6 million live cells each into 15–20 mL fresh BCH869 expansion medium in ultra-low attachment T75 flasks.
19. Exchange with fresh media on days 3 or 4 by following Step 13. Thereafter, exchange with fresh media every 2–3 days until ready to passage. Cell culture growth can be assessed by the change in media color to an orange color. If the media is yellowish, this is an indication that the cell density is too high and that exchanges with fresh media are warranted along with a larger total volume of fresh media.
20. Passage every 7–8 days following Step 14 and seed approximately 6 million cells per ultra-low attachment T75 flasks.

Staining Protocol

Start with high numbers of neurospheres in the T75 flask. To avoid sticking to the tube's wall, keep all solutions at 2–8 °C except for the 2nd antibody incubation.

1. Collect the suspension culture. Centrifuge at 1200 rpm (220 x g) for 5 minutes. Aspirate.
2. Wash the cell pellet once in 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate
3. Resuspend the cell pellet in 4% paraformaldehyde. Incubate at room temperature for 45 minutes to 1 hour.
4. Wash with 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate
5. Repeat step 4 two more times. Aspirate
6. Resuspend in 1X PBS containing 0.5% Triton® X-100. Incubate at room temperature for 1–2 hours with shaking.
7. Centrifuge at 1200 rpm for 5 minutes. Aspirate. Directly resuspend in Cyto Q Immuno Diluent & Block (NB307, Innovex Biosciences®) containing the antibody (1:100 dilution). Incubate at 4 °C overnight.
8. Next day, add 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate.
9. Repeat step 8 three more times.
10. Centrifuge at 1200 rpm for 5 minutes. Resuspend in Cyto Q Immuno Diluent & Block (NB307, Innovex Biosciences®) containing the 2nd antibody (1:500 dilution). Incubate at room temperature for 2 hours. Then on ice for 15 min before centrifuging.
11. Add 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate.
12. Repeat step 11 three more times. Aspirate.
13. Carefully aspirate the supernatant until the last 50–100 µL of PBS. Carefully resuspend in 200 µL DAPI and avoid causing bubbles. Transfer to 8-well chamber slide for confocal imaging. Mix the Dapi-cell suspension with Matrigel® to increase the viscosity for confocal scanning.

Cryopreservation of Cells

BCH869 Human H3K27M Glioma cells may be frozen in BCH869 Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Jones, C., Karajannis, M. A., Jones, D. T., Kieran, M. W., Monje, M., Baker, S. J., & Weiss, W. A. (2017). Pediatric high-grade glioma: biologically and clinically in need of new thinking. *Neuro-oncology*, 19(2), 153–161.
2. Gojo, J., Pavelka, Z., Zapletalova, D., Schmook, M. T., Mayr, L., Madlener, S., & Sterba, J. (2020). Personalized treatment of H3K27M-mutant pediatric diffuse gliomas provides improved therapeutic opportunities. *Frontiers in Oncology*, 9, 1436.
3. Pal, S., Kozono, D., Yang, X., Fendler, W., Fitts, W., Ni, J., & Haas-Kogan, D. A. (2018). Dual HDAC and PI3K inhibition abrogates NFκB-and FOXM1-mediated DNA damage response to radiosensitize pediatric high-grade gliomas. *Cancer research*, 78(14), 4007–4021.
4. Suvà, M. L., Rheinbay, E., Gillespie, S. M., Patel, A. P., Wakimoto, H., Rabkin, S. D., & Bernstein, B. E. (2014). Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. *Cell*, 157(3), 580–594.

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