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Data Sheet

BCH245 Human H3K27M Glioma Cell Line

Cancer Cell Line

SCC219

Pack Size ≥ 1x10^6 viable cells/vial

Store at: Liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Glioma, a type of tumor that arises in the brain and spinal cord and originates in the supportive glial cells surrounding neurons, is one of the most common types of primary brain tumors. Low-grade gliomas are the predominant glioma diagnosis among children, with very good prognosis and extremely rare malignant progression. Conversely, high-grade gliomas (HGG) are aggressive cancers and considered one of the most lethal tumors affecting the pediatric age group.[1] Diffuse midline gliomas (DMG) with histone H3 lysine27-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.[2]

BCH245 cell line is a patient-derived model of H3K27M glioma.[3] BCH245 cells grow as neurospheres in suspension culture and have been characterized via expression of epidermal growth factor receptor (EGFR). BCH245 cells have demonstrated utility in unraveling the mechanisms of glioma tumorigenesis and physiology,[4] representing a clinically relevant model for this especially devastating type of glioma.

Source

BCH245 was derived from diffuse midline glioma in pons tissue of a male pediatric patient.[3]

Short Tandem Repeat

D3S1358: 18	D18S51: 14	TPOX: 8, 12
D7S820: 8, 10	D5S818: 12	CSF1PO: 12
vWA: 14, 15	D13S317: 11	Amel: X, Y
FGA: 19	D16S539: 11	Penta D: 12
D8S1179: 11, 13	TH01: 9	Penta E: 14
D21S11: 31.2		

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.

Merck

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 interspecies contamination from mouse, rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

BCH245 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

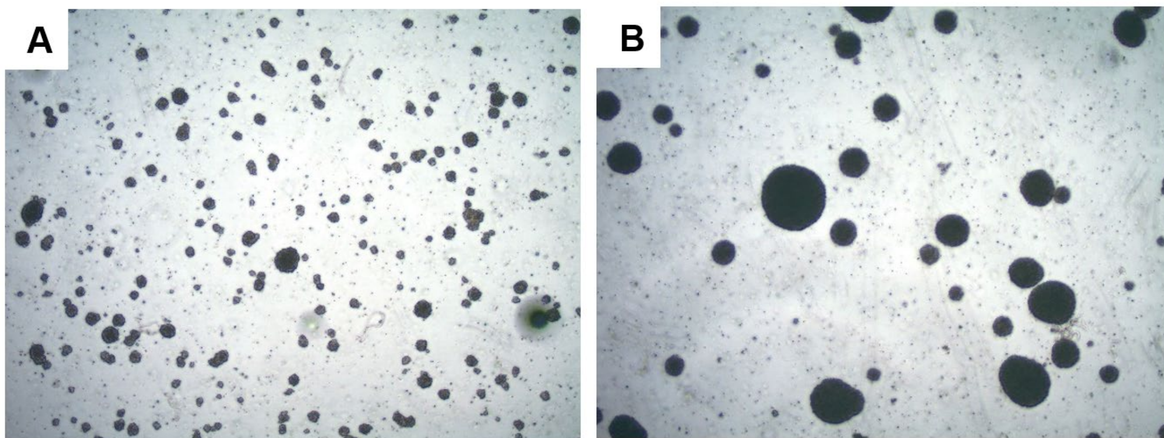

Figure 1. Bright-field images of BCH245 Human H3K27M glioma cells in culture, three (**A**) and seven (**B**) days after thaw in an ultra-low attachment T75 flask.

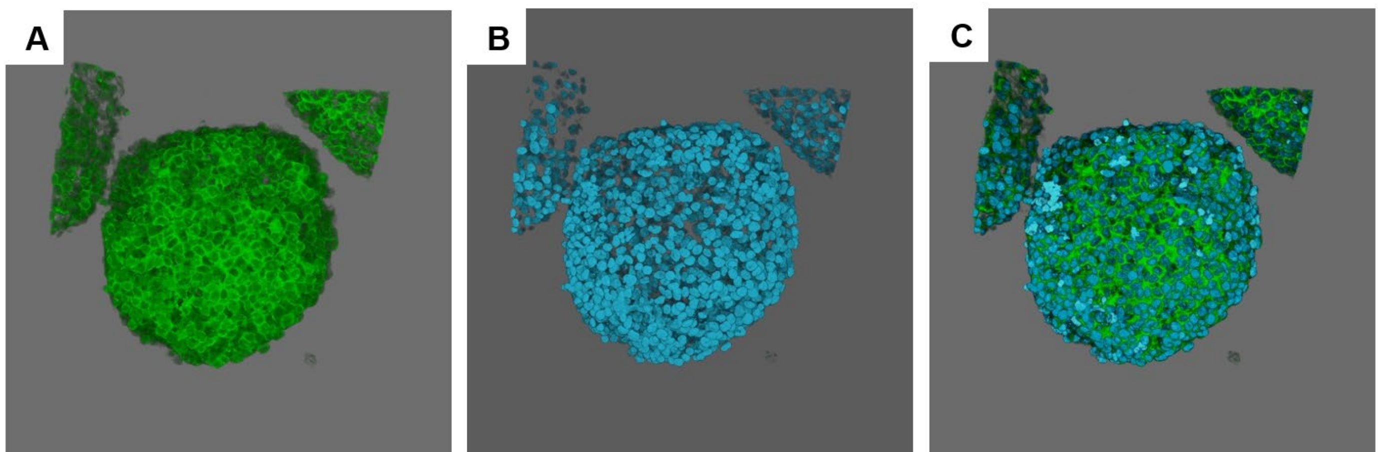

Figure 2. 3D images of BCH245 Human H3K27M glioma cells. Cells express EGFR (**A**, **C**, green). Nuclei were stained with Dapi (**B**, blue). Merged images (**C**).

Protocols

BCH245 Human H3K27M glioma cells grow as neurospheres and should be cultured on ultra-low attachment T75 flasks (Corning Cat. No. 3814). 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 (trituration). Do not use trypsin to dissociate the cells as this will result in loss of viability.

1. Prepare BCH245 Expansion Medium (500 mL).

   Note: All items may be purchased at SigmaAldrich.com unless otherwise noted.

Component	Quantity	Final Conc.	Cat. No
NeuroCult™ NS-A Proliferation Kit (Human)	500 mL		05751 (StemCell Technologies)
EGF, 20 mg/mL stock (1000X)	500 mL	20 ng/mL	01-107
FGF-2, 20 mg/mL stock (1000X)	500 mL	20 ng/mL	GF003AF-100UG
Heparin (10,000 U/mL stock)	50 mL	1 U/mL	375095-100KU
Antibiotic-Antimycotic Solution (100X); optional	5 mL	1X	A5955-100ML

2. Remove the vial of frozen BCH245 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 BCH245 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 BCH245 Expansion Medium.
10. Transfer the cell mixture to an ultra-low attachment T75 flask (Corning Cat. No. 3814).
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.
12. Because of the instability of the media supplements and growth factors, BCH245 neurosphere culture should be fed every 2-3 days.

For media exchanges

Transfer neurosphere suspension to a 15 mL or 50 mL conical tube. Pellet the neurospheres in a centrifuge at 220-300 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 (Corning Cat. No. 3814).

Passaging BCH245 neurospheres as single cell suspension

Note: Do not use trypsin to dissociate the cells. BCH245 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.

BCH245 neurospheres should be passaged every 7-8 days. Cells are dissociated to single cell suspension by mechanical trituration. Immediately after passage, refresh media on day 3 or 4. Thereafter, exchange with fresh media every 2 to 3 days until ready to passage.

1. Collect neurospheres and centrifuge at 220-300 x g for 5 minutes. Aspirate, leaving behind a little media.
2. Using a P1000 pipette (**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.
3. **Live cell count with Trypan Blue:** Take 5 mL of single cell suspension and resuspend in 45 mL culture medium. Add 50 mL Trypan Blue (Final 20X dilution). Count number of live cells using hemacytometer.
4. Plate approximately 3 million live cells each into 15-20 mL fresh BCH245 expansion medium in ultra-low attachment T75 flasks.
5. Exchange with fresh media on days 3 or 4 following For media exchanges step. 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.
6. Passage every 7-8 days following Passaging BCH245 neurospheres as single cell suspension step and seed approximately 3 million cells per ultra-low attachment T75 flasks.

Staining Protocol

Start with high numbers of neurospheres in the T75 flask. To avoid neurospheres 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 @ 220 x g for 5 minutes. Aspirate.
2. Wash the cell pellet once in 1X PBS. Centrifuge @ 220 x g for 5 minutes. Aspirate.
3. Resuspend the cell pellet in 4% paraformaldehyde. Incubate at room temperature for 45 min to 1 hour.
4. Wash with 1X PBS. Centrifuge @ 220 x g 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 220 x g for 5 minutes. Aspirate. Directly resuspend in Cyto Q™ Immuno Diluent & Block (Innovex Cat. No. NB307) containing the antibody (1:100 dilution). Incubate at 4 °C overnight.
8. Next day, add 1X PBS. Centrifuge at 220 x g for 5 minutes. Aspirate.
9. Repeat step 8 three more times.
10. Centrifuge at 220 x g for 5 minutes. Resuspend in Cyto Q™ Immuno Diluent & Block (Innovex Cat. No. NB307) 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 220 x g for 5 minutes. Aspirate.
12. Repeat step 11 three more times. Aspirate.
13. Carefully aspirate the supernatant, leaving 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

BCH245 Human H3K27M Glioma cells may be frozen in BCH245 Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Sci Rep 2020, 10(1): 8368.
2. Front Oncol 2020, 9:1436.
3. Science 2018, 360(6386): 331-335.
4. Nat Commun 2019, 10(1): 1262.

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