Product sheet

Cytion

NCH644 Cells | 300124

General information

Description	The NCH644 cell line is a glioblastoma stem-like cell line derived from patient tumors that lack EGFR amplification, making it a valuable model for studying glioblastoma biology, especially in the context of growth factor signaling and stem cell properties. Studies have demonstrated that in NCH644 cells, basic fibroblast growth factor (bFGF) plays a significant role in mediating growth and maintaining stem cell characteristics, whereas epidermal growth factor (EGF) does not show similar effects. NCH644 cells respond to bFGF by increasing the expression of stem cell markers such as CD133 and nestin, and they also exhibit enhanced resistance to apoptosis. This resistance, coupled with the lack of EGFR amplification, makes NCH644 a suitable model for understanding glioblastoma stem-like cell behavior, particularly under different growth factor conditions. Another notable feature of NCH644 is its slower proliferation rate when compared to other glioblastoma stem-like cell lines, such as NCH421k. However, when stimulated by bFGF, NCH644 cells show increased expression of EGFR, even in the absence of EGFR amplification, which highlights the interaction between fibroblast growth factor receptors (FGFRs) and EGFR signaling pathways. Moreover, bFGF plays a role in increasing the clonogenicity and multipotency of NCH644 cells, further supporting the notion that bFGF is crucial for maintaining the glioma stem-like properties of these cells. NCH644 cells have also been shown to harbor label-retaining, slow-cycling subpopulations that exhibit increased tumorigenicity and resistance to treatments such as irradiation and temozolomide. This subpopulation of label-retaining cells within the NCH644 line is highly tumorigenic, capable of forming tumors in immunocompromised mice even with small cell numbers. These features, combined with their resistance to standard treatments, make NCH644 a critical tool for investigating therapeutic strategies targeting glioblastoma stem cells.
Organism	Human
Tissue	Brain
Disease	Glioblastoma

Characteristics

Age	66 years
Gender	Female
Ethnicity	Caucasian
Growth properties	Spheroid culture

Regulatory Data

Citation	NCH644 (Cytion catalog number 300124)
Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_x914

Biomolecular Data

Antigen expression	Highly CD133 positive
Tumorigenic	Yes
Ploidy status	Aneuploid

Handling

Culture Medium	DMEM:Ham's F12 (1:1), w: 3.1 g/L Glucose, w: 2.5 mM L-Glutamine, w: 15 mM HEPES, w: 0.5 mM Sodium pyruvate, w: 1.2 g/L NaHCO3 (Cytion article number 820400a)
Supplements	Supplement the medium with 10% FBS, 5 mg/L Heparin, 20 ng/mL bFGF, 20 microgram/L EGF, 5 mg/L Insulin, 100 mg/L Transferrin, 5,2 microgram/L Na-selenit, 6,3 microgram/L Progesteron, 161,1 microgram/L Putrescin, 50 mg/L Hydrocortinson
Subculturing	For subculturing spheroid cultures, begin by mechanically dissociating the spheroids through pipetting up and down 5 to 10 times using an Eppendorf pipette with 1000 µl filter tips. After this, centrifuge the mixture at 300g for 5 minutes at room temperature to pellet the cells. Discard the supernatant and resuspend the cell pellet in fresh culture medium. Finally, transfer the resuspended cells into new culture vessels to promote further spheroid formation. This approach ensures efficient spheroid breakdown and readies them for continued growth in a new environment.
Seeding density	2×10^5 cells/ml
Fluid renewal	2 to 3 times per week
Post-Thaw Recovery	After thawing allow the cells to recover from the freezing process for at least 24 to 48 hours.

Freeze medium	As a cryopreservation medium, we use 50% basal medium + 40% FBS + 10% DMSO, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.
Thawing and Culturing Cells	1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required. 3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains. 4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening. 5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently. 6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. 7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth. 8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.
Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.
Shipping Conditions	Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.
Storage Conditions	For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

Sterility	Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods. To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.