Product sheet

NCH421K Cells | 300118

Description	NCH421K is a human glioblastoma stem-like cell line derived from a primary glioblastoma tumor obtained from an adult patient. This cell line belongs to a class of tumor-initiating cells that retain key features of neural stem cells, including self-renewal capacity, multipotency, and the ability to recapitulate tumor heterogeneity. NCH421K cells are typically cultured under serum-free conditions and grow as non-adherent neurospheres, a hallmark of stem-like glioma cultures. They express canonical stem cell markers such as CD133 and nestin, supporting their classification as a glioblastoma stem-like model. NCH421K exhibits growth and survival that are strongly dependent on basic fibroblast growth factor (bFGF), which promotes proliferation and maintenance of stem-like characteristics, whereas epidermal growth factor (EGF) has minimal effect on its expansion. The cells maintain high expression of stem cell markers under bFGF stimulation and demonstrate the ability to form tumors in vivo, highlighting their tumorigenic potential. Due to these properties, NCH421K is widely used in studies of glioblastoma stem cell biology, therapeutic resistance, differentiation strategies, and the evaluation of targeted treatments aimed at eradicating tumor-initiating cell populations. This cell line was established by Christel Herold-Mende from glioblastoma tissue.
Organism	Human
Tissue	Brain
Disease	Glioblastoma
Synonyms	NCH421k

Age	66 years
Gender	Male
Ethnicity	Caucasian
Growth properties	Spheroid culture

Citation	NCH421K (Cytion catalog number 300118)

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Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_x910
Tumorigenic	Yes
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
Doubling time	35 to 40 hours
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	1 to 2 x 10^5 cells/ml
Fluid renewal	2 to 3 times per week
Post-Thaw Recovery	Please 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.

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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.

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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.