

## NCH644 Cells | 300124

### General information

<b>Organism</b>	Human
<b>Tissue</b>	Brain
<b>Disease</b>	Glioblastoma

### Characteristics

<b>Age</b>	66 years
<b>Gender</b>	Female
<b>Ethnicity</b>	Caucasian
<b>Growth properties</b>	Spheroid culture

### Identifiers / Biosafety / Citation

<b>Citation</b>	NCH644 (Cytion catalog number 300124)
<b>Biosafety level</b>	1
<b>Depositor</b>	C. Herold-Mende

### Expression / Mutation

<b>Antigen expression</b>	Highly CD133 positive
<b>Tumorigenic</b>	Yes
<b>Ploidy status</b>	Aneuploid

### Handling

<b>Culture Medium</b>	DMEM:Ham's F12, w: 3.1 g/L Glucose, w: 1.6 mM L-Glutamine, w: 15 mM HEPES, w: 1.0 mM Sodium pyruvate, w: 1.2 g/L NaHCO <sub>3</sub> (Cytion article number 820400a)
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<b>Medium supplements</b>	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 Hydrocortison
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<b>Subculturing</b>	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.
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<b>Split ratio</b>	A ratio of 1:2 to 1:5 is recommended
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<b>Seeding density</b>	2 x 10 <sup>5</sup> cells/ml
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<b>Fluid renewal</b>	2 to 3 times per week
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<b>Freezing recovery</b>	After thawing allow the cells to recover from the freezing process for at least 24 to 48 hours.
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<b>Freeze medium</b>	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)
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#### Handling of cryopreserved cultures

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. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
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.

## Quality control / Genetic profile / HLA

#### 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.

#### STR profile

**CSF1PO:** 12  
**D13S317:** 10,13  
**D16S539:** 12,13  
**D5S818:** 9,10  
**D7S820:** 12,13  
**TH01:** 6,7  
**TPOX:** 8,11  
**vWA:** 15,19