Product sheet

NCH644 | 300124

General information

Organism	Human
Tissue	Brain
Disease	Glioblastoma

Characteristics

Age	66 years
Gender	Female
Ethnicity	Caucasian
Growth properties	Spheroid culture

Identifiers / Biosafety / Citation

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

Expression / Mutation

Antigen expression	Highly CD133 positive
Tumorigenic	Yes
Ploidy status	Aneuploid

Handling

Culture Medium	DMEM:Ham's F12

Medium supplements	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, 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 NaHCO3
Passaging solution	The utilization of a passaging solution is not necessary when passaging cells that are cultured in suspension. The appropriate procedure is to dilute the cells in accordance with the indicated guidelines.
Subculturing	Mechanical dissociation by pipetting (5-10 times) with an Eppendorf pipette and 1000 µl tips after centrifugation of the cells (10min, 300g, 10 degree Celsius). Subculture as soon as the cell clusters have reached an average diameter of 200 µm or the cell concentration has reached 1 x 10^6 cells/ml. It is recommended to distribute the cells into new flasks containing fresh medium thus diminishing the amount of dead cells and cell debris.
Split ratio	A ratio of 1:2 to 1:5 is recommended
Seeding density	2 x 10^5 cells/ml
Fluid renewal	2 to 3 times per week
Freezing recovery	After thawing allow the cells to recover from the freezing process for at least 24 to 48 hours.
Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)
Handling of cryopreserved cultures	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300 x g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.
Handling of proliferating cultures	One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility	Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.
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