Product sheet

HROG02 growing culture | 330931

General information

Description	This is one cell line of a series of tumor cell lines which have been established by PD Dr. Michael Linnebacher from Primary CRC resection specimens since 2006.
Organism	Human
Tissue	Brain, R, parietooccipital
Disease	Glioblastoma (grade IV)

Characteristics

Age	68 years
Gender	Male
Ethnicity	Caucasian
Morphology	A mixture of fibroblast-likes and epithelial-like cells
Growth properties	Adherent

Identifiers / Biosafety / Citation

Citation	HROG02 (Cytion catalog number 300931)
Biosafety level	1
Depositor	M. Linnebacher

Expression / Mutation

Antigen expression	HLA-A02+, ICAM-1 + Beta-microglobulin +, HLA-E+, HLA-G -, MIC A +, MIC-B -, GFAP+ , nestin +, vimentin +, S-100+, GBM+, BTSC+
Mutational profile	IDH 1 & 2 wt, TP53R248Q, 4q12(PDGFRA) amplified, K-Ras wt, B-RAFwt, PTEN-

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Handling

Culture Medium	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 NaHCO3 (Cytion article number 820400a)
Medium supplements	Supplement the medium with 10% FBS
Passaging solution	Accutase
Doubling time	36 to 54 hours
Subculturing	Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add TrypLE Express (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at 37 degree Celsius for 10 to 15 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium. This cell line will result in single cell suspension.
Split ratio	A ratio of 1:3 to 1:6 is recommended
Seeding density	1 x 10^4 cells/cm^2
Fluid renewal	Every 3 to 5 days
Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)
Handling of cryopreserved cultures	HROG02 cells are shipped in a deep-frozen state on dry ice. Upon receipt, confirm that the vial remains frozen. For storage, place the cryovial immediately at temperatures below -150 degrees. If you plan to culture the cells immediately, swiftly thaw the vial by shaking it in a 37 degrees water bath with clean water and an antimicrobial agent for 40-60 seconds. Remove the vial once a small ice clump persists, ensuring it remains cold. Proceed with all subsequent steps under aseptic conditions. In a sterile flow hood, disinfect the cryovial with 70% ethanol. Then, gently open the vial and transfer the cell suspension into a 15 ml centrifuge tube pre-filled with 8 ml of room temperature culture medium. Gently mix the cells. For cell separation, centrifuge at 300 x g for 3 minutes and dispose of the supernatant. Skipping centrifugation is optional, although any residual freezing medium should be removed after 24 hours. Resuspend the pellet gently in 10 ml of fresh culture medium and divide between two T25 culture flasks. Follow the subculture protocol for subsequent steps.

Handling of proliferating cultures	One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in a 50 ml centrifuge tube. 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. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Examine cell morphology and confluency using a microscope. Finally, incubate the flasks at 37 degrees Celsius for at least 24 hours.

Quality control / Genetic profile / HLA

Sterility	Mycoplasma contamination is rigorously 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.

HLA alleles	**A***: 01:01, 02:01 **B***: 08:01, 13:02 **C***: 06:02, 07:01 **DRB1***: 03:01, 07:01 **DRB4***: 01:03 **DQA1***: 02:01, 05:01 **DQB1***: 02:01, 02:02 **DPA1***: 01:03 **DPB1***: 04:01