

## HROG33 T0 M1 Cells | 300878

### General information

#### Description

HROG33 T0 M1 is a primary human glioblastoma multiforme (GBM) cell line established from freshly resected tumor tissue of an adult female patient with WHO grade IV glioblastoma located in the left occipitotemporal region. The designation “T0” refers to the primary tumor at initial diagnosis, and “M1” denotes the corresponding in vitro model derived from this specimen. The cell line was generated as part of a systematic effort to establish ultra-low passage GBM cultures from both fresh and vitally cryopreserved tumor material, with the aim of preserving patient-specific molecular and functional characteristics.

HROG33 T0 M1 exhibits adherent growth with a fibroblast-like morphology typical of primary GBM cultures. Cells form a monolayer and display consistent proliferative capacity in vitro. In the comparative establishment study, paired cultures derived from fresh and cryopreserved tumor tissue showed no significant differences in morphology, growth kinetics, or drug responsiveness. Immunophenotypic characterization of representative HROG cell lines demonstrated expression of neural lineage-associated markers including glial fibrillary acidic protein (GFAP), nestin, and vimentin, consistent with a glioma-derived phenotype. Molecular analyses performed across the HROG series included assessment of MGMT promoter methylation, EGFR amplification, and mutational status of TP53, IDH1/2, KRAS, and BRAF, supporting the retention of tumor-specific genomic features in established cultures.

Functionally, HROG-derived cell lines have been evaluated for sensitivity to standard-of-care and investigational agents used in GBM therapy, including temozolomide, BCNU (carmustine), vincristine, and imatinib. Drug response profiles of matched cell line pairs indicated stable and reproducible pharmacologic behavior following tissue cryopreservation. As an ultra-low passage primary GBM model, HROG33 T0 M1 provides a clinically relevant in vitro system for investigating glioblastoma biology, therapeutic response prediction, and patient-specific tumor heterogeneity while minimizing artifacts associated with long-term continuous cell line adaptation.

**Organism** Human

**Tissue** Brain

**Disease** Glioblastoma

### Characteristics

**Age** 46 years

**Gender** Female

**Ethnicity** Caucasian

**Growth properties** Adherent

**HROG33 T0 M1 Cells | 300878****Regulatory Data**

|                             |   |
|-----------------------------|---|
| <b>Citation</b>             | HROG33 T0 M1 (Cytion catalog number 300878) |
| <b>Biosafety level</b>      | 1   |
| <b>NCBI_TaxID</b>           | 9606  |
| <b>CellosaurusAccession</b> | CVCL_4U48                                   |

**Biomolecular Data****Handling**

|                             |   |
|-----------------------------|---|
| <b>Culture Medium</b>       | 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 NaHCO <sub>3</sub> (Cytion article number 820400a) |
| <b>Supplements</b>          | Supplement the medium with 10% FBS  |
| <b>Dissociation Reagent</b> | Accutase  |

**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

**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  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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  $300 \times g$  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

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  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  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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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.