

## HROC300 T2 M1 Cells | 300866

### General information

#### Description

HROC300 T2 M1 is a human colorectal carcinoma cell line derived from a primary tumor specimen resected from an adult patient within the HROC (Hansestadt Rostock Colorectal Cancer) model collection. The designation “T2” indicates that the tumor was obtained at a second surgical time point, while “M1” denotes the corresponding in vitro model established from this specimen. The HROC platform integrates comprehensive biobanking with standardized generation of patient-derived xenografts (PDX) and permanent low-passage cell lines, enabling molecularly annotated tumor models from consecutive colorectal cancer cases.

Establishment of HROC300 T2 M1 followed a standardized protocol involving mechanical dissociation of freshly resected tumor tissue, filtration to obtain single-cell suspensions, and seeding onto collagen-coated culture plates in defined tumor cell culture medium supplemented with glutamine, antibiotics, and antimycotics. Across the HROC cohort, permanent primary cell lines were generated from approximately 13% of attempted colorectal carcinoma samples, with successful establishment correlating in univariate analysis with higher tumor grading and advanced nodal status. Multivariate analysis identified nodal involvement as an independent predictor of successful in vitro model establishment. These findings reflect the enrichment of biologically aggressive phenotypes among successfully adapted cultures.

Within the broader HROC collection, models encompass all major molecular subtypes of colorectal carcinoma, including chromosomal instability (CIN), CpG island methylator phenotype (CIMP), microsatellite stable (MSS), and microsatellite instability-high (MSI-H) tumors, as well as diverse mutational backgrounds affecting genes such as KRAS, BRAF, TP53, APC, and PIK3CA. HROC300 T2 M1 was generated in this rigorously annotated context, allowing integration with matched clinicopathological data and, where available, corresponding PDX material. As a low-passage, patient-derived colorectal carcinoma model, HROC300 T2 M1 is suited for studies of tumor biology, genotype-phenotype associations, and preclinical therapeutic testing within a precision oncology framework.

**Organism** Human

**Tissue** Colorectal

**Disease** Adenocarcinoma, TNM stage T4aN1bM1R2L0V1, grading G2, Lk(n) + 3,  $\Sigma$  Lk(n) 22

**Metastatic site** Distant metastasis present (TNM M1; stage T4aN1bM1R2)

**Applications** CRC research; MSS colorectal adenocarcinoma; HROC Linnebacher biobank; drug sensitivity; high-stage CRC modeling; tumor biology

### Characteristics

**Age** 73 years

**Gender** Male

**HROC300 T2 M1 Cells | 300866****Ethnicity** Caucasian**Morphology** Epithelial-like**Cell type** Epithelial cells**Growth properties** Adherent**Regulatory Data****Citation** HROC300 T2 M1 (Cytion catalog number 300866)**Biosafety level** 1**NCBI\_TaxID** 9606**CellosaurusAccession** CVCL\_VQ94**GMO Status** No genetic modification; wildtype patient-derived CRC cell line (HROC Linnebacher biobank).**Biomolecular Data****MSI-status** MSS**Handling****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 NaHCO<sub>3</sub> (Cytion article number 820400a)**Supplements** Supplement the medium with 10% FBS**Dissociation Reagent** 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.

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**Fluid renewal**      Every 3 to 5 days

**Freeze medium**      As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

### 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.

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### 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.

## Quality Control & Molecular Analysis

### 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.