

**HROC278Met1 T2 M2 Cells | 300836**

**General information**

<b>Description</b>	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.
<b>Organism</b>	Human
<b>Tissue</b>	Peritoneal Metastasis, Established from a patient-derived xenograft of metastasis of primary CRC tissue (Colon ascendens, TNM stage T4N2M1R0L1V1, grade G3, Lk(n) +19, Σ Lk(n) 29)
<b>Disease</b>	Adenocarcinoma

**Characteristics**

<b>Age</b>	76 years
<b>Gender</b>	Female
<b>Ethnicity</b>	Caucasian
<b>Morphology</b>	Epithelial-like
<b>Growth properties</b>	Adherent

**Identifiers / Biosafety / Citation**

<b>Citation</b>	HROC278Met1 T2 M2 (Cytion catalog number 300836)
<b>Biosafety level</b>	1
<b>Depositor</b>	M. Linnebacher

**Expression / Mutation**

<b>Protein expression</b>	PTEN
<b>Tumorigenic</b>	Yes, in immune-suppressed nude mice
<b>Viruses</b>	Free of human pathogenic viruses SV40, JC/BK, HBV, HCV, HIV.

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**Mutational profile** B-RAFV600E APCwt, p53wt, K-Raswt, N-Raswt, H-Raswt, PIK3CAwt

### 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 NaHCO<sub>3</sub> (Cytion article number 820400a)

**Medium supplements** Supplement the medium with 10% FBS

**Passaging solution** Accutase

**Doubling time** 29 hours

**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.

**Split ratio** A ratio of 1:3 to 1:6 is recommended

**Seeding density**  $2 \times 10^4$  cells/cm<sup>2</sup>

**Fluid renewal** Every 3 to 5 days

**Freezing recovery** Few days

**Freeze medium** CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

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### Handling of cryopreserved cultures

HROC278Met1 T2 M2 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.

## 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.

### STR profile

**Amelogenin:** x,x  
**CSF1PO:** 10  
**D13S317:** 10  
**D16S539:** 13  
**D5S818:** 13  
**D7S820:** 11,12  
**TH01:** 7,9  
**TPOX:** 11  
**vWA:** 14,18