

**HROC69 Cells | 300828**

**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>	Colon ascendens, UICC IIIa
<b>Disease</b>	Primary adenocarcinoma, TNM stage T3N0M1R0L0V0, grading G3, Lk(n) + 0, ? Lk(n) 34
<b>Synonyms</b>	HROC69P

**Characteristics**

<b>Age</b>	62 years
<b>Gender</b>	Male
<b>Ethnicity</b>	Caucasian
<b>Morphology</b>	Epithelial-like
<b>Growth properties</b>	Adherent

**Identifiers / Biosafety / Citation**

<b>Citation</b>	HROC69 (Cytion catalog number 300828)
<b>Biosafety level</b>	1
<b>Depositor</b>	M. Linnebacher

**Expression / Mutation**

<b>Protein expression</b>	Beta-actin, osteopontin -, Toll-like receptor (TLR) 3 -, TLR4 moderate, TLR7 low, TLR8 -, PTEN
<b>Antigen expression</b>	High level phosphatidylserin (PS) expression

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<b>Tumorigenic</b>	Yes, in immune-suppressed nude mice
<b>Viruses</b>	Free of human pathogenic viruses SV40, JC/BK, HBV, HCV, HIV.
<b>Ploidy status</b>	Aneuploid
<b>Mutational profile</b>	APCR1450*, p53R306*, K-Raswt, B-RAFwt, N-Raswt, H-Raswt, SNP rs12628 codon 27, PIK3CAwt
<b>Handling</b>	
<b>Culture Medium</b>	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)
<b>Medium supplements</b>	Supplement the medium with 10% FBS
<b>Passaging solution</b>	Accutase
<b>Doubling time</b>	25 to 40 hours
<b>Subculturing</b>	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.
<b>Split ratio</b>	A ratio of 1:3 to 1:10 is recommended
<b>Seeding density</b>	2 x 10 <sup>4</sup> cells/cm <sup>2</sup>
<b>Fluid renewal</b>	Every 3 to 5 days
<b>Freezing recovery</b>	2 weeks
<b>Freeze medium</b>	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

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#### Handling of cryopreserved cultures

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. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
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.

### Quality control / Genetic profile / HLA

#### 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.

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**STR profile**

**Amelogenin:** x,y  
**CSF1PO:** 10,11  
**D13S317:** 11,12  
**D16S539:** 12,13  
**D5S818:** 12,13  
**D7S820:** 10,12  
**TH01:** 7,9.3  
**TPOX:** 8,12  
**vWA:** 17,19  
**D21S11:** 29,33.2