

## HROC296 Cells | 300853

## 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** Colon ascendens, UICC lia

**Disease** Primary adenocarcinoma, TNM stage T3N0M0R0L0V0, grading G2, Lk(n) +0,  $\Sigma$  Lk(n) 35

## Characteristics

**Age** 92 years

**Gender** Female

**Ethnicity** Caucasian

**Morphology** Epithelial-like

**Growth properties** Adherent

## Regulatory Data

**Citation** HROC296 (Cytion catalog number 300853)

**Biosafety level** 1

**NCBI\_TaxID** 9606

**CellosaurusAccession** CVCL\_1V02

## Biomolecular Data

**Antigen expression** CD326+

**Viruses** Free of human pathogenic viruses SV40, JC/BK, HBV, HCV, HIV.

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## 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
<b>Doubling time</b>	29 hours
<b>Subculturing</b>	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. It is recommended to use collagen-coated flasks.
<b>Seeding density</b>	$2 \times 10^4$ cells/cm <sup>2</sup>
<b>Fluid renewal</b>	Every 3 to 5 days
<b>Post-Thaw Recovery</b>	Fast
<b>Freeze medium</b>	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.

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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.