

HROC324 Cells | 300838

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 coecum, UICC IIIb
Disease	Primary adenocarcinoma, TNM stage T3N2M0R0L1V0, grading G3, Lk(n) +5, ? Lk(n) 24

Characteristics

Age	55 years
Gender	Female
Ethnicity	Caucasian
Morphology	Epithelial-like
Growth properties	Adherent

Identifiers / Biosafety / Citation

Citation	HROC324 (Cytion catalog number 300838)
Biosafety level	1
Depositor	M. Linnebacher

Expression / Mutation

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

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Mutational profile k-RasA59T, N-Raswt, H-Raswt, PIK3CAwt, B-Rafwt, MSH6 c.3417AC,p.Lys1140Gln (Exon5)

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 NaHCO3 (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 1:4 to 1:8 is recommended

Seeding density 2×10^4 cells/cm²

Fluid renewal Every 3 to 5 days

Freezing recovery Fast

Freeze medium 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°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°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,x
CSF1PO: 12
D13S317: 8,9
D16S539: 11
D5S818: 11
D7S820: 8,9
TH01: 8,9
TPOX: 8,11
vWA: 16,18

HLA alleles

A*: 03:01:01
B*: 07:02:01
C*: 07:02:01
DRB1*: 13:02:01G
DQA1*: 01:02:01
DQB1*: 06:04:01G
DPB1*: 02:01:02
E: 01:03:02