Product sheet

HROC39 T0 M2 | 300821

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 IIb, Established from a patient-derived xenograft of primary CRC tissue (Colon ascendens, TNM stage T4N0M0R0L0V1, grading G3, Lk(n) + 0, Σ Lk(n) 34).
Disease	Adenocarcinoma
Synonyms	HROC39x

Characteristics

Age	69 years
Gender	Male
Ethnicity	Caucasian
Morphology	Epithelial-like
Growth properties	Adherent

Identifiers / Biosafety / Citation

Citation	HROC39 T0 M2 (Cytion catalog number 300821)
Biosafety level	1
Depositor	M. Linnebacher

Expression / Mutation

Protein expression	PTEN

Antigen expression	Her2/neu +, EGFR +
Tumorigenic	Yes, in immune-suppressed nude mice
Viruses	Free of human pathogenic viruses SV40, JC/BK, HBV, HCV, HIV.
Ploidy status	Aneuploid
Mutational profile	APCmut, K-Raswt, N-Ras wt, H-Raswt SNP rs12628 at codon 27, B-Rafwt , p53 wt, PIK3CAwt

Handling

Culture Medium	DMEM:Ham's F12
Medium supplements	10% FBS, 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
Passaging solution	Accutase
Doubling time	38 hours
Subculturing	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.
Split ratio	A ratio of 1:4 to 1:8 is recommended
Seeding density	2 x 10^4 cells/cm^2
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)

Handling of cryopreserved cultures	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300 x g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.
Handling of proliferating cultures	One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility	Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.
STR profile	**Amelogenin**: x (Patient male, Y lost) **CSF1PO**: 12,13 **D13S317**: 12,13 **D16S539**: 11,12 **D5S818**: 12,13 **D7S820**: 12 **TH01**: 7,10 **TPOX**: 8,11 **vWA**: 16,18 **D21S11**: 29