Designation: **HROC32 T3 M1**

CLS order number: Cryovial: 300819
Vital: 330819

Origin and General Characteristics	
Depositor:	Michael Linnebacher
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	82 years
Gender:	Female
Tissue:	Colon ascendens, UICC IV
Morphology:	Epithelial
Cell type:	Established from a PDX (patient-derived xenograft) primary CRC tissue (Colon ascendens, TNM stage T4N2M1R0L0V1 grading G2; Lk(n) + 9, ∑ Lk(n) 14)
Growth Properties:	Adherent, in colonies
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. This cell line was derived from a late stage tumor of HROC32.
Culture Conditions and Handling	
Culture Medium:	DMEM/Ham's F12 with L-glutamine medium supplemented with 3 mM L-glutamine and 10% fetal bovine serum.
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°C 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 1:3 to 1:5 is recommended
Seeding density:	$2x10^4$ cells/cm^2
Fluid Renewal:	1 to 2 times weekly
Doubling time:	30h
Freeze Medium:	CM-ACF (CLS order number 800650, 50ml)
Freezing recovery:	1-2 weeks
Sterility:	Mycoplasma specific PCR: negative; Mycoplasma specific PlasmoTest: negative; Bacteria, fungi: negative.
Biosafety Level:	1
Special Features of the Cell Line	
Tumorigenic:	Yes, in immuno-suppressed nude mice
Viruses:	Free of human pathogenic viruses SV40, JC/BK, HBV, HCV, HIV.
Molecular type:	spSTD, ß-catenin ^{Translocation}, CIMP $^{not\ methylated}$, CIMP-number: 0
MSI-status:	MSS
Phenotype:	CIN^{pos}
Ploidy status:	aneuploid

Tumor marker secretion:	CA19-9^{low}, CEA^{high}, IL-8, IL-10^{-}	
Cell marker:	CD15 $^{+}$, CD24 $^{+}$, CD44 $^{+}$, CD55 $^{+}$, CD58 $^{+}$, CD50 $^{+}$, CD 54 $^{+}$, CD66acde $^{+}$, CD71 $^{+}$, CD102 $^{+}$, CD326 $^{+}$, CD80 $^{-}$, CD86^{-}, EpCAM $^{+}$, HLA-A2 $^{+}$, MHC I $^{+}$, MHC II $^{+}$ (IFN-γ pretreated); Her2/neu $^{+}$,	
DNA Profile (STR):	Amelogenin: X,X CSF1PO: 14 D13S317: 11,12 D16S539: 11,12 D5S818: 11,12	D7S820: 8,11 THO1: 8,9 TPOX: 8,11 vWA: 19 D21S11: 31
Mutational profile:	APC^{wt}, p53^{R282W}, K-Ras^{G12A}, N-Ras^{wt}, H-Ras^{wt} SNP rs12628 at codon 27, PIK3CAst, B-Raf^{wt}	
Protein expression:	PTEN	
Related Cell Lines:	HROC32, CLS Cat.-no. 300818, cryovial, 330818, proliferating culture. Bc HROC32	

References:

Linnebacher M., Maletzki C., Ostwald C., Klier U., Krohn M. , Klar E. and Prall F., Cryopreservation of human colorectal carcinomas prior to xenografting, BMC Cancer 2010, 10:362

Maletzki C., Gock M., Randow M., Klar E., Huehns M., Prall F., Linnebacher M. ; Establishment and characterization of cell lines from chromosomal instable colorectal cancer; World Journal of Gastroenterology January 7, 2015, Vol. 21, Issue1

Linnebacher M., Ostwald C., Koczan D., Salem T., Schneider B., Krohn M., Ernst M. and Prall F.; Single nucleotide poymorphism array analysis of microsatellite-stable, diploid/near-diploid colorectal carcinomas withourt the CpG island methylator phenotype; ONCOLOGY LETTERS 5: 173-178, 2013

Medico E. et al. 30. Apr 2015. Nature Communications 6:7002 | DOI: 10.1038/ncomms8002

Recommendations for handling of adherent cell cultures following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) must be stored in liquid nitrogen (-196°C) or at least at -80°C after arrival.

If immediate culturing is intended, please follow these instructions:

Quickly thaw by rapid agitation in a 37°C 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. Note: 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 300xg 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 10ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the Subculture section.

Proliferating Cultures

The cell culture flasks are completely filled with cell culture medium to prevent loss of cells during transit. Remove the entire medium except for a sufficient volume to cover the floor of the flask. Incubate at 37°C for 24 hrs.

Sometimes the cultures are handled roughly during transit, and most of the cells detach and float in the culture medium. If this has occurred remove the entire content of the flask and centrifuge at 300x g for 5 minutes. Take off the supernatant, resuspend the cells in 10 ml of culture medium and transfer the entire cell suspension into cell culture flasks of suitable size (do not seed in more than 1T75 flask).

Safety precautions for frozen cell lines

If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:

- Protective gloves and clothing should be used and a facemask or safety goggles must be worn when storing and/or thawing the cryovial.
- The removal of a cryovial from liquid nitrogen can result in the explosion of the cryovial creating flying fragments.

References: Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.