Designation: **HROC43**

CLS order number: Cryovial: 300823
Vital: 330823
gDNA: 300823GD05
Cell Pellet: 300823CP

Origin and General Characteristics	
Depositor:	Michael Linnebacher
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	72 years
Gender:	Male
Tissue:	Colon ascendens; UICC IIIb
Morphology:	Epithelial
Cell type:	Primary adenocarcinoma, TNM stage T3N2M0R0L1V0, grading G3; Lk(n) + 30, ∑ Lk(n) 36
Growth Properties:	Adherent, in colonies
Description:	This is one of a series of tumor cell lines which have been established by PD Dr. Michael Linnebacher from Primary CRC resection specimens since 2006.
References:	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. Maletzki C., Huehns M. Knapp P., Waukosin N., Klar E., Prall F., Linnebacher M; Functional Characterization and Drug Response of Freshly Established Patient-Derived Tumor Models with CpG Island Methylator Phenotype PLoSOne, 10(11):e0143194, 2015. doi: 10, 1371/journal, pone.0143194.
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:	27h
Freeze Medium:	CM-ACF (CLS order number 800650, 50ml)
Freezing recovery:	1 week
Sterility:	Mycoplasma specific PCR: negative; Mycoplasma specific PlasmoTest: negative; Bacteria, fungi: negative.

Biosafety Level:	1
Safety precautions:	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 transferring frozen samples into or removing from the liquid nitrogen tank. The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments. 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.
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:	CIMP-L, non MSI, CIMP-number: 3, ß-catenin Translocation -
DNA Profile (STR):	Amelogenin: X (Patient male, Y lost) CSF1PO: 11 D13S317: 8,12 D16S539: 9,13 D5S818: 11,12 D7S820: 13 THO1: 10 TPOX: 11 vWA: 15,17 D21S11: 31.2
	Deletions in the Y-chromosome sometimes cause a misidentification of the biological sample as female. However the cell line is still called authenticated.
Ploidy status:	Aneuploid
DNA Methylation marker:	$MLH1^{-}$, $CDKN2A^{+}$, $NEUROG1^{+}$, $CRABP1^{+}$, $CACNA1G^{-}$, $MGMT^{-}$, $IGF2^{-}$, $SOCS2^{-}$, $RUNX3^{+}$
MSI-status:	MSS
Cell Marker:	$CD326^{+}$, $CD44^{+}$, $CD15^{+}$, $CD71^{+}$, $CD73^{+}$, $CD274^{+}$, $CD47^{+}$, $CD54^{+}$, $CD95^{+}$, $CD276^{+}$, $CD133^{-}$, $CD66acde^{weak}$, IDO^{+}, $cFLIP^{+}$, MHC-I^{+}, $MHCII^{weak}$ after IFN-y treatment, $EpCAM^{+}$
Mutational profile:	APC^{Q1429*}, $p53^{S241fs*5}$, K-Ras^{mut}, N-Ras^{wt}, H-Ras^{wt}, $PIK3CA^{wt}$, B-Raf^{wt}
Tumor marker secretion:	CA19-9^{high}, CEA^{high}, IL-8, IL-10 $^{-}$, IL-6^{-}, TGF-ß$^{-}$, TGF-□$^{-}$
Protein expression:	PTEN
Related Cell Lines:	B-LCL-HROC43, CLS catalog-no. 302067

Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.

Recommendations for handling of adherent cell cultures following delivery	
Cryopreserved cells	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(s) must be stored below -150°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, 2xT25, come filled with cell culture medium. Collect the entire medium in 2x 50 ml centrifuge tubes. Carefully add 5 ml of cell culture medium to each of the two T25 cell culture flasks. Control the cell morphology and confluency under the microscope. Incubate at 37°C for a minimum of 24 hrs. Spin down the collected medium at 300x 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 1xT25 cell culture. Incubate at 37°C for a minimum of 24 hrs.

Warranty:	CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed.
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