Designation: **HROC46 T0 M1**

CLS order number: Cryovial: 300824
Vital: 330824
gDNA: 300824GD05
Cell pellet: 300824CP

Origin and General Characteristics	
Depositor:	Michael Linnebacher
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	66 years
Gender:	Male
Tissue:	Colon ascendens, UICC IV
Morphology:	Epithelial
Cell type:	Established from a PDX (patient-derived xenograft) of primary CRC tissue (Colon ascendens, TNM stage T3N0M1R2L0V1, grading G3, Lk(n) + 0, ∑ Lk(n) 34)
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.
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 Medico E. et al. 30. Apr 2015. Nature Communications 6:7002 | DOI: 10.1038/ncomms8002
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. Following this procedure will result in a 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:	25 to 40 h
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
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:	spSTD, CIMP-number: 0
MSI-status:	MSS
Cell Marker:	CD274 $^{+}$, CD197 $^{+}$, EpCAM $^{+}$, CD40 $^{+}$, CD253 $^{+}$, CD56 $^{+}$, CD44 $^{+}$, CD66acde $^{+}$, CD50 $^{-}$, CD58 $^{-}$, CD178 $^{-}$, CD86 $^{-}$
Mutational profile:	APC^{mut}, K-Ras^{G12V}, N-Ras^{wt}, H-Ras ^{wt}, p53^{wt}, PIK3CA^{wt}, B-Raf^{wt}
Ploidy status:	aneuploid
Protein expression:	PTEN
Related Cell Lines	B-LCL-HROC46 (Synonym: Bc HROC46), CLS catalog no. 302068.

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	If immediate culturing is not intended, the cryovial(s) must be stored below -150°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 (size (do not seed in more than 1T75 flask).

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.

Disclaimer:	The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term ‘Commercial use’ shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale. This product from CLS has been manufactured under license for third parties. The customer shall take the submitted licensing terms and conditions of third parties into consideration and may only make use of such within the scope of the rights granted therein.