Designation: **CC531**

CLS order number: Cryovial: 500387
Vital: 550387

Origin and General Characteristics	
Depositor:	Dr. Peter J.K. Kuppen, LUMC
Organism:	WAG rats
Tissue:	Colon
Cell type:	Carcinoma
Growth Properties:	Monolayer, adherent
Description:	The original tumor CC531 was induced by Dr. Richard Marquet from Rotterdam, the Netherlands using dimethylhydrazin in Wag rats (a Wistar derived strain). The tumor was for some time maintained as a transplantable tumor. The cell line was established from this tumor as described by Thomas et al. (1993).
References:	Marquet RL, Westbroek DL, Jeekel J. Interferon treatment of a transplantable rat colon adenocarcinoma: importance of tumor site. INt J Cancer 33: 689-692, 1984. Thomas C, Nijenhuis AM, Timens W, Kuppen PJ, Daemen T, Scherphof GL. Liver metastasis model of colon cancer in the rat: immunohistochemical characterization. Invasion-Metastasis 13(2):102-12, 1993. Hagenaars M, Ensink NG, Basse PH, Hokland M, Nannmark U, Eggermont AM, van de Velde CJ , Fleuren GJ and Kuppen PJ. The microscopic anatomy of experimental rat CC531 colon tumour metastases: consequences for immunotherapy? Clin Exp Metastasis 18: 189-196, 2000a. Hagenaars M, Koelomij R, Ensink NG, van Eendenburg JD, van Vlierberghe RL, Eggermont AM, van de Velde CJ , Fleuren GJ and Kuppen PJ. The development of novel mouse monoclonal antibodies aganst the CC531 rat colon adenocarcinoma. Clin Exp Metastasis 18: 281-289, 2000b.
Culture Conditions and Handling	
Culture Medium:	RPMI 1640 medium supplemented with 4 mM L-glutamine, buffered with 20 mM HEPES and 12 mM $NaHCO_3$, and 10% fetal bovine serum (MG-73, CLS order number 820703, ready-to-use, or MG-73a, CLS order number 820703a, basic, without FBS).
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 Accutase (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperatures for 10 minutes. Carefully resuspend the cells, the addition of medium is optional but not necessary, and dispense into new flasks which contain fresh medium.
Split Ratio:	A ratio of 1:5 to 1:10 is recommended
Seeding density:	1-2x10^4 cells/cm^2 will result in a confluent monolayer within 3-4 days.
Fluid Renewal:	1 to 2 times weekly
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Freezing recovery:	After thawing, plate the cells at 5 x 10^4 cells/cm^2 and allow the cells to recover from the freezing process and to adhere for at least 24 hrs.
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: 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 nude mice, syngeneic WAG-Rij rats
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR

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.
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 of CLS has been

		manufactured under license for third parties.