Designation: **LS-513**

CLS order number: Cryovial: 300457
Vital: 330457

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	63 years
Gender:	male
Tissue:	Colorectal carcinoma, cecum, Dukes' type C
Morphology:	Epithelial
Growth Properties:	Monolayer, adherent
Description:	The colorectal carcinoma cell line LS-513 was isolated in 1985 from a primary tumor biopsy of a 63 year old Caucasian male patient. He was diagnosed with a Dukes' C mucin secreting cecal tumor located at the Bauhin valve. LS-513 cells express the major histocompatibility (MHC) class I antigens HLA and beta 2 microglobulin. MHC class II antigens (HLA-DR, DQ, and DP were not detected). TGF beta-1 is inhibitory for proliferation of LS-513 cells, whereas TGF beta-2 has no effect on the growth of these cells. LS-513 cells are 100-fold less sensitive to TGF beta-1 than the LS-1034 (ATCC CRL-2158) cell line. LS-513 cells are multidrug resistant (MDR) and are tumorigenic in nude mice. Colony forming efficiency was 30% in methylcellulose.
Culture Conditions and Handling	
Culture Medium:	Ham's F12 medium supplemented with L-glutamine and 10% fetal bovine serum (MG-60, CLS order number 820600).
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 ambiente temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split Ratio:	A ratio of 1:3 to 1:4 is recommended
Fluid Renewal:	2 times weekly
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1
Special Features of the Cell Line	
Tumorigenic:	yes, forms tumors in nude mice
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR
Karyotype:	Two stem lines can be distinguished. The main one was represented in 65% of the cells, with a modal number of 51,XY and 3 markers, M1 - der(1)t(1;15), M2 - der(2)t(2;3)der(3)t(2;3), M3, and a monosomy 15. The second stem line had a modal chromosome number of 52,XY and presented M2 and M3 plus an isochromosome for the long arm of chromosome 1 called M4. A trisomy 5,7, a tetrasomy 13, and a monosomy 2 and 3 were present in all of the cells analyzed; the line did not exhibit monosomy 15.
DNA Profile (STR):	Amelogenin: X ,Y CSF1PO: 10,10 D13S317: 9,10 D16S539: 12,13 D5S818: 11 D7S820: 8,11
	vWA: 16,17 D3S1358: 15 D21S11: 30 D18S51: 12,18 Penta E: 5,18 Penta D: 9,14

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	THO1: 8 TPOX: 8	D8S1179: 13 FGA: 19,21
Oncogene:	p53 wt	
Antigen Expression:	Carcinoembryonic antigen (CEA); ICAM-1; HLA class I positive	
Protein Expression:	CEA + (50%), p53 +	
Products:	Transforming growth factor beta 1 (TGF beta-1, 83 pg per 10 exp6 cells per 24 hours)	
References: Suardet L et al. Responsiveness of three newly established human colorectal cancer cell lines to transforming growth factors beta 1 and beta 2. Cancer Res 52: 3705-12, 1992.		

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.