Designation: **NCI-H209**

CLS order number: Cryovial: 300183
Vital: 330183

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Gender:	Male
Tissue:	Lung; from metastatic site: bone marrow
Morphology:	Epithelial
Cell type:	Small cell lung carcinoma
Growth Properties:	Aggregates in suspension
Description:	The NCI-H209 cell line was derived by A.F. Gazdar and associates in 1979 from the bone marrow of a patient with small cell cancer of the lung. The bone marrow specimen was taken prior to therapy. The line is a classic SCLC cell line which expresses elevated levels of four biochemical markers (neuron specific enolase, brain isoenzyme of creatine kinase, L-DOPA decarboxylase and bombesin-like immunoreactivity. C-myc DNA sequences are not amplified. No gross structural DNA abnormalities were detected. This is a cell line that grows as large aggregates in suspension. Only the aggregates are viable, but no meaningful viability percentage can be measured. The medium will normally contain large amounts of cell debris. The cells express an aberrant form of RB1 that is not phosphorylated, apparently due to a single point mutation at codon 706 (Cys -> Phe).
References:	Moody TW et al. Bombesin-like peptides in small cell lung cancer: biochemical characterization and secretion from a cell line. Life Sci 32: 487-93, 1983.
Culture Conditions and Handling	
Culture Medium:	RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum (MG-70, CLS order number 820700).
Subculturing:	The line should be subcultured by dilution with fresh medium. Alternatively, the clusters may be collected by centrifugation and resuspended in fresh medium.
Seeding density:	$1x10^5$/ml
Split Ratio:	A ratio of 1:2 to 1:3 is recommended
Fluid Renewal:	2 to 3 times weekly
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Freezing recovery:	After thawing, allow the cells to recover from the freezing process for at least 48 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; forms transplantable tumors with typical SCLC histology in nude mice	
Oncogene:	pRB (RB1, abnormal)	
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR	
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11 D13S317: 11 D16S539: 9,12 D5S818: 12 D7S820: 9 THO1: 7,9 TPOX: 8	vWA: 18,19 D3S1358: 18 D21S11: 32.2 D18S51: 13 Penta E: 11,12 Penta D: 11,12 D8S1179: 12,13 FGA: 20,24
Protein Expression :	p53 negative	
Isoenzymes:	G6PD, B; PGM1, 1-2; PGM3, 1; ES-D, 1; Me-2, 0; AK-1, 1; GLO-1, 1-2; Phenotype Frequency Product = 0.0624	
Products:	The line produces normal amounts of p53 mRNA relative to normal lung.	

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

Recommendations for handling of cells growing in suspension 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 one T25 cell culture flask. All further steps are described in the Subculture section.
Proliferating Cultures	The cell culture flask, 1xT25, comes filled with cell culture medium. Incubate at 37°C for a minimum of 24 hrs. Count the cells, spin down the cell suspension at 300x g for 3 minutes to collect the cells. Resuspend the cells in an appropriate amount of fresh cell culture medium and transfer to new cell culture flasks. 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.