Designation: **NCI-H82**

CLS order number: Cryovial: 300442
Vital: 330442

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	40 years old
Gender:	Male
Tissue:	Lung (pleural effusion)
Morphology:	Round cells in cluster
Cell type:	Small cell carcinoma
Growth Properties:	Aggregates in suspension; the cells grow in very large aggregates, and the aggregates are the only viable cell population
Description:	The NCI-H82 cell line was derived by A.F. Gazdar and associates in 1978 from the pleural fluid of a patient with small cell cancer of the lung. The morphology of the original tumor was not characteristic of SCLC. The line is a biochemical and morphological variant of SCLC that expresses neuron specific enolase and the brain isoenzyme of creatine kinase.
References:	Gazdar AF et al. Levels of creatine kinase and its BB isoenzyme in lung cancer specimens and cultures. Cancer Res 41: 2773-7, 1981.
Culture Conditions and Handling	
Culture Medium:	RPMI 1640 medium supplemented with 4.5g/L glucose, 2mM L-glutamine, and 10% fetal bovine serum (MG-72, CLS order number 820702).
Subculturing:	This line grows as aggregates of cells in suspension. Sub-culture by transferring the cell suspension into new cell culture flasks already filled with the appropriate volume of fresh cell culture medium. Alternatively, the cells may be collected by centrifugation and dispersed into fresh medium.
Split Ratio:	A ratio of 1:2 to 1:5 is recommended
Seeding density:	1×10^5 cells/ml
Fluid Renewal:	2 to 3 times weekly
Doubling time:	About 25h
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Freezing recovery:	Following thawing, the cells need at least 48h for recovery from the freezing process.
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 non-typical SCLC histology in nude mice	
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR	
Karyotype:	This is a near triploid human cell line. The modal chromosome number is 58, occurring at 44% with polyploidy at 3%. Marker chromosomes der(1)t(1;709p13;p11), t(13q;?HSR;15q) and der(190t(19;?)(q13.4;?) were common to most cells. There were two distinct subpopulations readily distinguished by karyotype. Besides uniform changes in the numbers of copies of some normal chromosomes, one population had der(3)t(3;20)(p11;p11?), t(3q19p), i(7q) and a minute chromosome of unknown origin. The other had t(1q17p), del(1)(q21), der(3)t(3;7)(p12;q11) plus two other markers. Each cell had two copies of a normal X chromosome. The Y chromosome was not detected in Q banded preparations.	
DNA Profile (STR):	Amelogenin: X,X CSF1PO: 11 D13S317: 8 D16S539: 12 D5S818: 12 D7S820: 10,13 THO1: 9,9,3 TPOX: 11	vWA: 14 D3S1358: 17 D21S11: 28,30 D18S51: 14,18 Penta E: 11,12 Penta D: 10,12 D8S1179: 13 FGA: 24,25
Ploidy status:	Aneuploid	
MSI-status:	Stable (MSS)	
Oncogene:	C-myc DNA sequences are amplified about 25 fold; there is a 24 fold increase in c-myc RNA relative to normal cells. There is expression of v-fes, v-fms, Ha-ras, Ki-ras, N-ras and c-raf 1 mRNAs.	
Cell Marker:	Insulin-like growth factor II receptor (IGF II); atrial natriuretic peptide (ANP); The cells are reported to express functional ANP receptors, but treatment with ANP does not alter their growth pattern. The cells stain positively for neurofilaments and vimentin. The cells do not have detectable levels of L-DOPA decarboxylase or bombesin.	
Tumor marker secretion:	The cells produce an abnormally sized p53 mRNA (3.7 kb).	
Isoenzymes:	G6PD, B; PGM1, 1-2; PGM3, 1-2; ES-D, 1; Me-2, 1; AK-1, 1; GLO-1, 1; Phenotype Frequency Product = 0.0082	
Protein expression:	p53 positive	

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.