

**Designation: SK-NEP-1**

CLS order number: Cryovial: 300341
Vital: 330341

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	25 years of age
Gender:	Female
Tissue:	Metastatic site: Pleural effusion
Cell type:	Reclassified as belonging to the Ewing sarcoma family.
Morphology:	Epithelial
Growth Properties:	Suspension
Description:	This cell line was established by G. Trempe and J. Fogh in 1971 from a metastatic site. Ultrastructural features include few microvilli, junctional complexes, well-formed Golgi, mostly smooth ER, lipid droplets, no virus particles.
References:	Fogh J et al. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J Natl Cancer Inst 58: 209-14, 1977.
Culture Conditions and Handling	
Culture Medium:	McCoy's 5a medium supplemented with L-glutamine and 10% fetal bovine serum (MG-200, CLS order number 821200).
Subculturing:	Cultures can be maintained by addition or replacement of fresh medium. Establish new cultures at 1×10^5 cells/ml and maintain at between 10^5 and 10^6 cells/ml.
Split Ratio:	A ratio of 1:2 to 1:4 is recommended
Seeding density:	1×10^5 /ml
Fluid Renewal:	Every 2 to 4 days
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
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.
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR
Karyotype:	(P12) hypotriploid to hypertriploid (+A1, +A2, +C, +D, +E, +F, +G) with abnormalities including acrocentric fragments, secondary constrictions and large sub telocentric markers

DNA Profile (STR):	Amelogenin: X,X CSF1PO: 10 D13S317: 11 D16S539: 11 D5S818: 13 D7S820: 8,10 THO1: 8,9.3 TPOX: 8,11	WWA: 15,19 D3S1358: 14,15 D21S11: 29,31 D18S51: 15,17 Penta E: 7,18 Penta D: 11,12 D8S1179: 12 FGA: 24
Antigen Expression:	Blood Type A; Rh+	
Isoenzymes:	PGM3, 1; PGM1, 1-2; ES-D, 1; Me-2, 2; AK-1, 1; GLO-1, 2; G6PD, B; Phenotype Frequency Product: 0.0029	
Genetics:	P53 mut	

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