Designation: **SCL-II**

CLS order number: Cryovial: 300497
Vital: 330497
gDNA: 300497GD, 5 µg
Whole cell lysate: 300497CL, 500 µg protein
Snap-frozen cell pellet: 300497CP, 1 Mio cells per pellet

Origin and General Characteristics	
Depositor:	DKFZ, Heidelberg
	CLS is official licensee of the HaCaT, HaCaT-ras A5, HaCaT-ras II-4, SCL-I and SCL-II cell lines on behalf of the DKFZ Heidelberg.
Organism:	*Homo sapiens* (human)
Ethnicity:	Caucasian
Age:	91 years old
Gender:	Male
Tissue:	Undifferentiated SCC (squamous cell carcinoma) from facial tissue
Cell type:	Undifferentiated squamous cell carcinoma cells
Growth Properties:	Monolayer, adherent
Description:	The human cell line SCL-II was established from an undifferentiated squamous cell carcinoma of a patient by Boukamp et al. in 1983.
References:	Tilgen W, Boukamp P, Breitkreuz D, Dzarlieva RT, Engstner M, Haag D and Fusenig NE. Preservation of Morphological, Functional, and Karyotypic Traits during Long-Term Culture and In Vivo Passage of Two Human Skin Squamous Cell Carcinomas. Cancer Res 43: 5995-6011, 1983.
Citation format:	If use of this cell line results in a scientific publication, it should be cited as: SCL-II (CLS Cell Lines Service, 300497).
Permit:	Signing a MTA is not necessary for the transfer of this material. Please cite the publication of the References section in every written communication.
Culture Conditions and Handling	
Culture Medium:	DMEM medium supplemented with 4.5g/L glucose, 2 mM L-glutamine and 10% fetal bovine serum (MG-30, CLS order number 820300).
Passaging solution:	TrypLE™ Express (Life Technologies). The protocol of the manufacturer should be followed..
Subculturing:	1. Remove the culture 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). 2. Add TrypLE™ Express, 1-2ml per T25, 2.5ml per T75 cell culture flask, the cell sheet must be covered completely, and incubate at 37°C for a maximum of 10 min. 3. Once the cells have detached, add cell culture medium, and resuspend the cells to end up with a single cell suspension. 4. Dispense into new flasks which contain fresh cell culture medium.
Split Ratio:	A ratio of 1:5 to 1:10 is recommended
Seeding density:	$1x10^4$ cells/cm^2
Fluid Renewal:	2 times weekly
Freeze Medium:	CM-1 (CLS order number 800150, 50ml)
Population doubling time:	About 40 to 50 hrs
Sterility:	Mycoplasma specific PCR: negative

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	Cell-based assay (Plasmotest): 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:	Formation of highly differentiated, locally invasive squamous cell carcinoma in Balb/c-nu/nu mice.	
Karyotype:	Aneuploid (hypodiploid, few metaphases hypotetraploid)	
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 8,12 D16S539: 10,11 D5S818: 9 D7S820: 8,12 TH01: 8 TPOX: 8,11	D3S1358: 14 D21S11: 29 D18S51: 17 Penta E: 13 Penta D: 9,13 D8S1179: 12,13 FGA: 26 vWA: 15,17
Protein expression:	P53 $^{(+)}$;	
SARS-COV-2 active proteins :	n.d.	
Related cell lines and derivatives:	SCL-I, CLS catalog no. 300496, gDNA, 300496GD, 5 µg, whole cell lysate 300496CL, 500 µg, snap-frozen cell pellet, 300496CP, 1 Mio cells/pellet (bulk preparations are available on request).	
Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.	
Recommendations for the handling after delivery		
Thawing of Frozen cells:	Quickly thaw by rapid agitation in a 37°C water bath within 1-3 minutes. 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 (a small ice clump should remain and the cryovial should still be cold). All operations from now on 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 resuspend the cells in 8 ml of cell culture medium***1** in a 15-ml-tube. Remove the cryoprotectant by centrifugation (300xg, 3 minutes). Carefully take off the supernatant, resuspend the cells in 10 ml of fresh medium and pipette into two T25 cell culture flasks. Incubate at 37°C / 5% CO_2. If the cells grow well and reach confluence (or post-confluence, optimum 3 to 4 days past confluence), split into T75 cell culture flask(s) containing 15 ml of medium.	
Delivered as proliferating culture:	For transport, the cell culture flasks have been completely filled with culture medium to prevent loss of cells during transit. Collect the entire medium in sterile 50 ml tubes except for 5 ml to cover the bottom of the flasks. Control if the cells adhere at the bottom of the flasks. If the cell sheet covers at least 50% of the bottom, discard the supernatant. If you notice lower confluence, continue with collecting the cells in the supernatant. Centrifuge the supernatant for 3 min at 300xg, remove the medium and resuspend the pellet in 5 ml cell culture medium. Transfer the 5 ml into 1x T25 cell culture flask.	

	Incubate the T25 cell culture flasks at 37°C/5% CO_2 for at least 24 hrs; as cells are stressed during transit, they might need an even longer recovery time of 48 hrs or more. Note: Even if the cells are delivered with > 90% confluence, let the cells recover from transit for 24 hrs.
Freezing procedure:	The cultures should be about 80-90% confluent and media changed one day ahead to stimulate proliferation. Under a sterile flow cabinet, detach the cells according to the protocol described above. Count the cells and collect the cells at 300xg for 3 minutes. Resuspend the cells at a concentration of 2 million/ml of freezing medium (e.g. CM-1, (CLS order number 800150, 50ml). Aliquot the cells in sterile cryovials (2 million/vial) and put them at -20°C for 40 minutes. Place the cryovials overnight at -80°C into a deep-freezer. For long term storage, transfer the frozen vials into a liquid nitrogen container. Storage of the frozen vials at -80°C for more than 2-3 days is not recommended.

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 from CLS has been manufactured under license for the DKFZ, Heidelberg, Germany. The customer shall take the submitted licensing terms and conditions of the MTA DKFZ into consideration and may only make use of such within the scope of the rights granted therein.