Designation: **HaCaT**

CLS order number: Cryovial: 300493
Vital: 330493

Origin and General Characteristics	
Depositor:	DKFZ, Heidelberg
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	62 years old
Gender:	Male
Tissue:	Skin
Cell type:	Keratinocyte
Growth Properties:	Monolayer, adherent
Description:	In vitro spontaneously transformed keratinocytes from histologically normal skin.
References:	Boukamp P, Dzarlieva-Petrusevska RT, Breitkreuz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J. Cell Biol. 106: 761-771, 1988.
Citation format:	If use of this cell line results in a scientific publication, it should be cited as: HaCaT (CLS Cell Lines Service, 300493).
Permit:	The MTA of the DKFZ is required for the transfer of this CLS material.
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:	The 1:1 mixture of EDTA (stock. 0.05%) and trypsin (stock: 0.1%) must be prepared each time ahead of detaching the cells using PBS without Ca^{2+} and Mg^{2+} to provide a physiologic osmolarity. Ready-to-use mixtures of trypsin/EDTA are not recommended, as this may result in cell clumps. As an alternative, TrypLE™ Express (Life Technologies) instead of trypsin/EDTA can be used. 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 freshly prepared 0.05% EDTA solution, 1-2ml per T25, 2.5ml per T75 cell culture flask, the cell sheet must be covered completely, and incubate at 37°C for 10 min. 3. Add freshly prepared trypsin/EDTA, 0.05%/0.025% solution *2 (1ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. The cells must detach within 1-2 minutes. 4. Stop the trypsin activity using FBS-containing cell culture medium. 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)
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:	No
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR
Karyotype:	Aneuploid (hypotetraploid)
DNA Profile (STR) 2015:	Amelogenin: X,X CSF1PO: 9,11 D13S317: 10,12 D16S539: 9,12 D5S818: 12 D7S820: 9,11 THO1: 9.3 TPOX: 11,12 vWA: 16,17 D3S1358: 16 D21S11: 28,30.2 D18S51: 12 Penta E: 7,12 Penta D: 11,13 D8S1179: 14 FGA: 24
Protein expression :	P53 positive, CEA positive

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

Recommendations for the handling of HaCaT cells	
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 (s 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; culture of adherent HaCaT cells.	For transport, the cell culture flasks have been completely filled with culture medium[*1] to prevent loss of cells during transit. Put the flasks into the incubator at 37°C/5% CO_2 without removal of the medium. 24 hrs later, take off the medium except for 5 ml (T25) or 15 ml (T75) and incubate for another 24 hrs. At post-confluence, optimum 3 to 4 days past confluence, 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). Add freshly prepared 0.05% EDTA solution, 1-2ml per T25, 2.5ml per T75 cell culture flask, the cell sheet must be covered completely, and incubate at 37°C for 10 min. Remove the EDTA, and add freshly prepared trypsin/EDTA, 0.05%/0.025% solution [*2] (1ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. The cells must detach within 1-2 minutes. Stop the trypsin activity using FBS-containing cell culture medium. Dispense into new flasks which contain fresh cell culture medium. Split the cells at a maximum ratio of 1:10 or a plating density of $1x10^4/cm^2$. The optimum time interval between passages is 7 to 10 days.

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 third parties. The customer shall take the submitted licensing terms and conditions of third parties into consideration and may only make use of such within the scope of the rights granted therein.