Product sheet

HaCaT Cells | 300493

General information

Description	HaCaT cells are a pivotal model in dermatological research, offering insights into the complex mechanisms of skin biology and pathology. The spontaneously immortalized HaCaT cell line is derived from adult human epidermal cells and retains the capacity to proliferate and undergo differentiation, similar to basal keratinocytes in vivo. HaCaT cells serve as a robust platform for investigating the epidermal differentiation process and studying the epidermal differentiation markers essential for maintaining skin integrity. The susceptibility of HaCaT cells to apoptosis and their sensitivity to apoptosis-inducing agents are extensively studied, particularly in the context of cytotoxic agents like RIPL. Researchers assess these agents' cytotoxicities and the extent of cytotoxicity using HaCaT cells, utilizing techniques such as fluorescence microscopy to visualize cellular changes. Researchers have leveraged HaCaT cells to examine the effects of various agents, including antimicrobial substrates and their influence on cell viability. These cells are an excellent substrate for testing antimicrobial biomaterials and antimicrobial atelocollagen substrates, crucial for skin repair and medical applications. The HaCaT epidermal line also plays a crucial role in studying cellular senescence, cytokines, and gene expression profiles related to aging and chronic diseases. The transcriptional profiles of HaCaT cells, including the role of κB and microRNAs, provide insight into the regulatory mechanisms at the molecular level. The HaCaT keratinocyte line, with their characteristics as epidermal keratinocytes, offers a tractable system for dissecting the intricate interplay between epidermal cells and the immune system, specifically the role of keratinocytes in disease states. They enable the exploration of epigenetic modifications and their influence on the differentiation of keratinocytes, including the formation of the cornified envelope, a key feature in the skin's barrier function. In summary, HaCaT cells are an indispensable model in dermatological research, facilitating a deeper understanding of skin biology and pathology through their resemblance to basal keratinocytes and their ability to undergo cell growth and differentiation. Their application spans from studying epidermal differentiation and antimicrobial effects to exploring cellular responses such as apoptosis, making them a cornerstone in cell biology and biomedical research.
Organism	Human
Tissue	Skin

Characteristics

Age	62 years
Gender	Male
Ethnicity	Caucasian
Cell type	Keratinocytes with a diameter of 20-25 micrometer.

Growth properties	Adherent

Identifiers / Biosafety / Citation

Citation	HaCaT (Cytion catalog number 300493)
Biosafety level	1
Depositor	DKFZ, Heidelberg

Expression / Mutation

Tumorigenic	No
Karyotype	Aneuploid (hypotetraploid)

Handling

Culture Medium	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
Medium supplements	Supplement the medium with 10% FBS
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 Ca2+ and Mg2+ 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.
Doubling time	The doubling time of HaCaT cells is 28 hours.
Subculturing	Discard the old medium and wash the adherent cells with PBS that lacks calcium and magnesium, using 3-5 ml for T25 flasks and 5-10 ml for T75 flasks. Add a freshly prepared 0.05% EDTA solution, ensuring complete coverage of the cell layer with 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Incubate at 37 degrees Celsius for 10 minutes. Then, add a freshly prepared trypsin/EDTA solution at concentrations of 0.05% and 0.025%, respectively, using 1 ml for T25 flasks and 2.5 ml for T75 flasks, again ensuring full coverage of the cells. The cells should detach within 1-2 minutes. Neutralize the trypsin by adding cell culture medium containing FBS. Finally, transfer the cell suspension into new flasks filled with fresh culture medium.
Split ratio	A ratio of 1:5 to 1:10 is recommended

Seeding density	1 x 10^4 cells/cm^2
Fluid renewal	2 times per week
Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

Handling of cryopreserved cultures

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150?C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37?C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Quality control / Genetic profile / HLA

Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.

STR profile	**Amelogenin**: x,x **CSF1PO**: 9,11 **D13S317**: 10,12 **D16S539**: 9,12 **D5S818**: 12 **D7S820**: 9,11 **TH01**: 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 **D1S1656**: 11,12 **D2S1338**: 17,25 **D12S391**: 18,23 **D19S433**: 13,14
HLA alleles	**A***: 01.01.1900 07:01 **B***: 01.01.1900 16:01, 02.01.1900 03:01 **C***: 03:04:01, 15:02:01 **DRB1***: 04:01:01, 15:01:01 **DQA1***: 01:02:01, 03:03:01 **DQB1***: 03:01:01, 06:02:01 **DPB1***: 03:01:01, 04:01:01 **E**: 01:03:01, 01:03:02