Product sheet

HaCaT-ras A5 Cells | 300494

General information

Description	HaCaT-ras A5 cells are a spontaneously immortalized, non-tumorigenic human skin keratinocyte cell line, instrumental in the study of tumour microenvironment interactions and the progression of skin carcinoma. Originating from a 62-year-old Caucasian male, these cells have undergone clonal selection and mutagenesis, which, coupled with autocrine growth factor regulation, enable the formation of slow-growing, highly differentiated benign cystic tumours in Balb/c-nu/nu mice. This makes them a valuable model for investigating the cellular dynamics and molecular mechanisms of tumour progression in vivo. The HaCaT-ras A5 cells are particularly useful for elucidating the complex interactions between tumour cells and surrounding stromal cells, including fibroblasts, immune cells, and endothelial cells. These interactions are mediated by the secretion of various signalling molecules such as growth factors, cytokines, and proteases, among which interleukin-6 (IL-6) plays a pivotal role. IL-6 is known to become dysregulated in many cancer types, primarily through overexpression or persistent activation of the STAT3 transcription factor. Research has shown that IL-6 stimulation of HaCaT-ras A5 cells significantly increases their proliferation via the JAK/STAT signalling pathway, while fibroblasts remain unaffected due to a more potent inhibition by SOCS3, a negative regulator of this pathway. This differential response has been captured in a mathematical model describing the dynamics of STAT3 and SOCS3, providing a deeper understanding of cell-specific signalling cascades. Furthermore, IL-6 not only directly affects HaCaT-ras A5 cell proliferation but also indirectly influences the cellular environment through the activation of a network of growth factors such as HGF, KGF, VEGF, and IL-8. Gene expression analysis involving over 16,000 genes revealed that IL-6 stimulation upregulates 19 genes related to the interferon signal pathway in both HaCaT-ras A5 cells and fibroblasts, which correlates with the observed growth inhibition in fibroblasts. The discovery of the crucial role of SerpinB4 in the proliferation of HaCaT-ras A5 cells, confirmed through siRNA knockdown experiments, underscores the intricate regulation by IL-6 in both tumour and stromal cells. This comprehensive understanding of IL-6's roles enhances the potential for developing targeted therapeutic strategies aimed at modulating IL-6 signalling pathways in the tumour microenvironment. Overall, HaCaT-ras A5 cells offer a robust model for exploring the complex interplay within the tumour microenvironment, paving the way for novel approaches in cancer research and therapy development.
Organism	Human
Tissue	Skin
Synonyms	HaCaT-ras clone A-5, HaCaT A-5, A-5, A5

Characteristics

Age	62 years
Gender	Male

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Ethnicity	Caucasian
Cell type	Keratinocyte
Growth properties	Adherent

Regulatory Data

Citation	HaCaT-ras A5 (Cytion catalog number 300494)
Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_xK16
GMO Status	GMO-S1: This HaCaT-ras A5 line contains a plasmid-borne c-Ha-ras oncogene construct for epithelial transformation research. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Protein expression	P53 (+), CEA (+),
Tumorigenic	Formation of benign tumors in Balb/c-nu/nu mice.
Karyotype	Aneuploid (hypotetraploid)

Handling

Culture Medium	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
Supplements	Supplement the medium with 10% FBS
Dissociation Reagent	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, TrypLETM Express (Life Technologies) instead of trypsin/EDTA can be used. The protocol of the manufacturer should be followed.

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Subculturing

1. **Discard Old Medium**: Remove the old medium from the flasks.
2. **Wash Cells**: Add 3-5 ml of PBS (without calcium and magnesium) to T25 flasks, or 5-10 ml to T75 flasks, to wash the adherent cells.
3. **Add EDTA Solution**: Cover the cell layer completely with a freshly prepared 0.05% EDTA solution-use 1-2 ml for T25 flasks and 2.5 ml for T75 flasks.
4. **Incubation**: Incubate the flasks at 37 degrees Celsius for 10 minutes.
5. **Add Trypsin/EDTA Solution**: Following the incubation, add a freshly prepared trypsin/EDTA solution (0.05% trypsin, 0.025% EDTA) to the flasks, ensuring the cells are fully covered-use 1 ml for T25 flasks and 2.5 ml for T75 flasks.
6. **Monitor Detachment**: Observe the cells, which should detach within 1-2 minutes.
7. **Neutralize Trypsin**: Add FBS-containing cell culture medium to stop the trypsin activity.
8. **Transfer Cells**: Dispense the cell suspension into new flasks pre-filled with fresh culture medium.

Seeding density	1 x 10^4 cells/cm^2
Fluid renewal	2 times per week
Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Thawing and Culturing Cells	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. 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.
Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.
Shipping Conditions	Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.
Storage Conditions	For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

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.