Product sheet

HaCaT-ras II-4 growing culture | 330495

General information

Description	HaCaT-ras II-4 cells are a remarkable and extensively studied cellular model in biological science. These cells are derived from spontaneously immortalized human skin keratinocytes, known as HaCaT cells, which were modified through transfection with the c-Ha-ras (EJ) oncogene. The selection of these cells was based on their resistance to G418, a selective antibiotic, as described in the comprehensive study conducted by Boukamp et al. in 1990. One notable characteristic of HaCaT-ras II-4 cells is their tumorigenic nature. When these clonal cells are injected into Balb/c-nu/nu mice, they exhibit a fascinating behaviour by forming highly differentiated and locally invasive squamous cell carcinomas. This unique property allows researchers to explore tumour development and progression mechanisms within a controlled experimental environment. HaCaT-ras II-4 cells are predominantly derived from the Caucasian population, ensuring relevance to a specific ethnic group in scientific investigations. Their origin and characteristics make them an invaluable resource for researchers interested in studying various skin biology and differentiation aspects. These cells possess a partially to fully differentiated phenotype under typical culture conditions. This phenotype is attributed to the abundant presence of calcium in both traditional media and fetal bovine serum, which provides an ideal environment for the cells to exhibit characteristics resembling those of mature skin cells. This feature allows researchers to investigate the intricate processes involved in skin development, wound healing, and epidermal differentiation. With their tumorigenic nature and the ability to replicate skin biology in vitro, HaCaT-ras II-4 cells offer a unique opportunity to explore the molecular pathways associated with skin cancer and other skin-related disorders. By utilizing this exceptional cellular model, researchers can gain deeper insights into the underlying mechanisms of tumorigenesis, invasive potential, and therapeutic interventions. HaCaT-ras II-4 cells are a vital tool for biological science research, specifically in skin biology and differentiation studies. Their origin from spontaneously immortalized human skin keratinocytes, modification with the c-Ha-ras (EJ) oncogene, and subsequent tumorigenic behaviour in mice make them invaluable for investigating skin-related diseases and therapeutic approaches. By harnessing the unique characteristics of HaCaT-ras II-4 cells, researchers can unlock a deeper understanding of skin biology and contribute to advancing medical knowledge and treatment options for various skin disorders.
Organism	Human
Tissue	Skin
Synonyms	HaCaT-ras clone II-4, HaCaT II-4, II-4

Characteristics

Age	62 years
Gender	Male
Ethnicity	Caucasian
Cell type	Keratinocyte

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Growth properties	Adherent

Identifiers / Biosafety / Citation

Citation	HaCaT-ras II-4 (Cytion catalog number 300495)
Biosafety level	1
Depositor	DKFZ, Heidelberg

Expression / Mutation

Protein expression	P53 (+), CEA (+),
Tumorigenic	Formation of highly differentiated, locally invasive squamous cell carcinoma in Balb/c-nu/nu mice.
Karyotype	Aneuploid (hypotetraploid)

Handling

Culture Medium	DMEM
Medium supplements	10% FBS, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO3, w: 1.0 mM Sodium pyruvate
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, TrypLETM Express (Life Technologies) instead of trypsin/EDTA can be used. The protocol of the manufacturer should be followed.
Subculturing	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 degree Celsius for 10 min. 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 ratio	A ratio of 1:5 to 1:10 is recommended

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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	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 must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius 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. 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 300 x g 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 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.
Handling of proliferating cultures	One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility	Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.

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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