HaCaT-ras II-4

300495

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.

Tissue	Skin

Synonyms

- HaCaT-ras clone II-4
- HaCaT II-4
- II-4

CLASSIFICATION

Organism	Human
Sex	Male
Growth properties	Adherent
GMO status	GMO-S1

GMO status details

GMO-S1: This human keratinocyte line (HaCaT-ras II-4) contains a plasmid encoding c-Ha-Ras oncogene sequences introduced by transfection, enabling transformed growth behavior. The construct is integrated into HaCaT-derived keratinocytes. This classification applies only within Germany and may differ elsewhere.

Biosafety level	1

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CHARACTERISTICS

Cell type	Keratinocyte
Tumourigenic	Formation of highly differentiated, locally invasive squamous cell carcinoma in Balb/c-nu/nu mice.

Karyotype

Aneuploid (hypotetraploid)

IDENTIFIERS

Citation

HaCaT-ras II-4 (Cytion catalog number 300495)

NCBI TaxID	9606
Cellosaurus	CVCL_3868

EXPRESSION AND SUSCEPTIBILITY

Protein expression

P53 (+), CEA (+),

HANDLING AND CULTURE

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)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

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.

Subculturing

Discard Old Medium: Remove the old medium from the flasks.
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.
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.
Incubation: Incubate the flasks at 37 degrees Celsius for 10 minutes.
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.
Monitor Detachment: Observe the cells, which should detach within 1-2 minutes.
Neutralize Trypsin: Add FBS-containing cell culture medium to stop the trypsin activity.
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

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Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

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.

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.

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.

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.

DONOR

Donor age	62 years
Ethnicity	Caucasian

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Third-party agreements apply	Yes