**Product sheet**

# HEL-299 Cells | 300193

## General information

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| **Description** | HEL-299 is a human lung fibroblast cell line derived from an adult individual. This cell line is particularly noted for its finite capacity to propagate in culture, typically entering senescence after approximately ten passages. This characteristic makes HEL-299 a useful model for studying cellular aging and senescence, as well as the dynamics of cell growth and replication under controlled conditions.<br><br>In addition to its applications in aging research, HEL-299 also serves as a model for studying signal transduction pathways. Specifically, it has been observed that the expression of the M2 muscarinic receptor in these cells is downregulated following stimulation with protein kinase C. This response highlights the cell line's utility in pharmacological research and in the investigation of mechanisms underlying receptor-mediated signaling and regulation. The alteration in receptor expression following kinase activity can provide insights into cellular responses to external stimuli, potentially aiding in the development of therapeutic strategies targeting similar pathways in various diseases. |
| **Organism** | Human |
| **Tissue** | Lung |
| **Synonyms** | HEL 299, Hel-299, Hel 299, HEL299 |

## Characteristics

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| **Age** | Fetus |
| **Gender** | Male |
| **Ethnicity** | African |
| **Growth properties** | Adherent |

## Regulatory Data

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| **Citation** | HEL-299 (Cytion catalog number 300193) |
| **Biosafety level** | 1 |
| **NCBI_TaxID** | 9606 |
| **CellosaurusAccession** | CVCL_2480 |

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## Biomolecular Data

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| **Receptors expressed** | M2 muscarinic receptor |
| **Protein expression** | P53 negative |
| **Isoenzymes** | G6PD, A |
| **Virus susceptibility** | Vesicular stomatitis (Indiana), poliovirus 1 |
| **Reverse transcriptase** | Negative |
| **Karyotype** | Normal human male, diploid, stable |

## Handling

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| **Culture Medium** | Ham's F12, w: 1.0 mM stable Glutamine, w: 1.0 mM Sodium pyruvate, w: 1.1 g/L NaHCO3 (Cytion article number 820600a) |
| **Supplements** | Supplement the medium with 10% FBS, 1 ng/mL bFGF |
| **Dissociation Reagent** | Accutase |
| **Subculturing** | Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium. |
| **Seeding density** | $1 \times 10^4$ cells/cm$^2$ |
| **Post-Thaw Recovery** | After thawing, plate the cells at $5 \times 10^4$ cells/cm$^2$ and allow the cells to recover from the freezing process and to adhere for at least 24 hours. |

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| **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.

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| **Incubation Atmosphere** | 37°C, 5% $CO_2$, humidified atmosphere. |
| **Flask Coating** | None |
| **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. |

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| **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

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| **Sterility** | Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.<br><br>To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections. |