**Product sheet**

# HEK293-CTLA4 Cells | 305423

## General information

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| **Description** | **Disclaimer: The prices displayed for cell lines are exclusively for academic/not-for-profit customers. For commercial entitites the price is approximately €6,250.**<br>**If you represent a commercial entity or are unsure which category applies, please contact us.**<br><br>The HEK293-CTLA4 cell line is a stable recombinant HEK293 cell line engineered to express the CTLA4 receptor at a medium-low level, approximately 4,000 molecules per cell. This cell line was developed using inscreenex's landing pad technology, ensuring precise and reproducible integration of the CTLA4 gene at a specific, pre-validated genomic locus. CTLA4, also known as CD152, is an immune checkpoint protein that plays a crucial role in regulating T-cell activation. By competing with the stimulatory receptor CD28 for binding to B7 molecules (CD80 and CD86) on antigen-presenting cells, CTLA4 downregulates immune responses, thus maintaining self-tolerance and preventing autoimmunity. CTLA4 is a key target in cancer immunotherapy, particularly for drugs like ipilimumab, which aim to enhance anti-tumor immunity by blocking this checkpoint and activating T cells.<br><br>The expression of CTLA4 in this cell line was confirmed using flow cytometry with a target-specific antibody, ensuring reliable and consistent receptor density across the cell population. |
| **Organism** | Human |
| **Tissue** | Fetal Kidney |
| **Disease** | Transformed/immortalized; non-tumorigenic (HEK293 background) |
| **Applications** | Checkpoint inhibitor research; CTLA4-targeted antibody development (ipilimumab, tremelimumab analogs); T-cell co-stimulation assays; immuno-oncology screening; bispecific antibody engineering |

## Characteristics

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| **Age** | Fetus |
| **Gender** | Female |
| **Morphology** | Epithelial-like |
| **Cell type** | Epithelial cells |
| **Growth properties** | Monolayer, adherent |

## Regulatory Data

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| **Citation** | HEK293-CTLA4 (Cytion catalog number 305423) |
| **Biosafety level** | 1 |
| **NCBI_TaxID** | 9606 |
| **CellosaurusAccession** | CVCL_6G20 |
| **GMO Status** | GMO-S1: This HEK293 variant contains a CTLA-4 expression construct for immune checkpoint research. This classification applies only within Germany and may differ elsewhere. |

## Biomolecular Data

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| **Receptors expressed** | CTLA4 (CD152) |

## Handling

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| **Culture Medium** | RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO3 (Cytion article number 820700a) |
| **Supplements** | Supplement the medium with 10% FBS, 1 mM sodium pyruvate, 10 mM HEPES, 1% NEAA. Add Geneticin (G418-Sulfat) to achieve a final concentration of 1 mg/mL. |
| **Dissociation Reagent** | Trypsin-EDTA |
| **Doubling time** | approx. 24-36 hours |
| **Subculturing** | For routine adherent cell culture: Aspirate the old culture medium from the adherent cells, and wash them with PBS to remove any remaining medium. After aspirating the PBS, add the appropriate volume of Trypsin/EDTA solution based on the culture vessel size (e.g., 1 ml for a T25 flask, 3 ml for a T75 flask) and incubate at room temperature or 37°C until the cells detach (5-10 minutes). Monitor detachment under a microscope, and gently tap the vessel if necessary to release the cells. Once detached, add complete medium to inactivate the Trypsin/EDTA, gently resuspend the cells, and transfer an aliquot of the cell suspension into a new culture vessel containing fresh medium. Place the vessel in an incubator set to 37°C with 5% $CO_2$, and change the medium every 2-3 days. |
| **Split ratio** | 1 to 5 |
| **Seeding density** | 2 to 4 x $10^4$ cells/$cm^2$ |

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| **Fluid renewal** | 2 to 3 times per week |
| **Post-Thaw Recovery** | After thawing, split the cells at a ratio of 1:2 to 1:3 in T25 flasks and allow the cells to recover from the freezing process and to adhere (for adherent cultures) for at least 24 hours. |
| **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.<br>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.<br>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.<br>4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.<br>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.<br>6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.<br>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.<br>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. |

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| **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

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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. |