

## HEK293-CTLA4 Cells | 305423

## General information

## Description

**Disclaimer: The prices displayed for cell lines are exclusively for not-for-profit customers. If you represent a commercial entity, please contact us for alternative pricing.**

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.

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

## Characteristics

**Age** Fetus

**Gender** Female

**Morphology** Epithelial-like

**Growth properties** Monolayer, adherent

## Regulatory Data

**Citation** HEK293-CTLA4 (Cytion catalog number 305423)

**Biosafety level** 1

**NCBI\_TaxID** 9606

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**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**

**Receptors expressed** CTLA4 (CD152)

**Handling**

**Culture Medium** RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO<sub>3</sub> (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

**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<sub>2</sub>, and change the medium every 2-3 days.

**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 at least 24 hours.

For best attachment and viability after thawing the cells, we recommend using Collagen-coated flasks or plates for the initial seeding after cryo-recovery. Collagen coating is not required for subsequent routine culture of the cells.

**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.

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### 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  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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  $300 \times g$  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

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , 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  $-78^{\circ}\text{C}$  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  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  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.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.