

**CTLA4 Ig-24 Cells | 305014**

**General information**

**Description**

CTLA4 Ig-24 cells, derived from an adult female Chinese hamster (*Cricetulus griseus*), are a spontaneously immortalized cell line which have been genetically modified by introducing the human CTLA-4 gene, resulting in the expression of a fusion protein.

The fusion protein possesses a dominant attribute of CTLA4Ig, making CTLA4 Ig-24 cells a unique and essential tool in immunology research. CTLA-4, a member of the immunoglobulin superfamily, is primarily expressed in activated T cells and acts as an inhibitory signal transmitter to regulate T cell function.

It shares homology with CD28, a T-cell co-stimulatory protein, and both molecules bind to CD80 (B7-1) and CD86 (B7-2) proteins on antigen-presenting cells. CTLA-4 demonstrates a greater affinity and avidity for CD80 and CD86 than CD28, allowing it to outcompete CD28 for binding to these ligands. By doing so, CTLA-4 transmits an inhibitory signal to T cells, while CD28 transmits a stimulatory signal.

This intricate regulatory mechanism is pivotal in maintaining immune balance and preventing excessive immune responses. Interestingly, CTLA-4 is also found in regulatory T cells (Tregs) and contributes to their inhibitory function.

When T cells are activated through the T cell receptor (TCR) and CD28, the expression of CTLA-4 increases. Furthermore, CTLA-4 may influence cell motility and signal signalling through the PI3 kinase pathway.

**Organism** Hamster

**Tissue** Ovary

**Synonyms** CTLA4Ig-24

**Characteristics**

**Gender** Female

**Morphology** Epithelial

**Growth properties** Adherent

**Identifiers / Biosafety / Citation**

**Citation** CTLA4 Ig-24 (Cytion catalog number 305014)

**Biosafety level** 1

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### Expression / Mutation

### Handling

**Culture Medium** DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO<sub>3</sub>, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)

**Medium supplements** Supplement the medium with 10% FBS, 0.2 mM proline, 0.001 mM methotrexate

**Passaging solution** 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.

**Split ratio** 1: 3 to 1: 4

**Fluid renewal** 2 to 3 times per week

**Freeze medium** CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

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#### Handling of cryopreserved cultures

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. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
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.

#### Quality control / Genetic profile / HLA

##### 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.