

Mouse Recombinant CTLA4 Stable Cell Line
Cat. No. M00570**Version 04282015****I. INTRODUCTION**

Catalog Number: M00570

Cell Line Name: CHO-K1/mouse CTLA4

Gene Synonyms: Cd152; Ly-56; Ctla-4

Expressed Gene: Codon Optimized from NM_009843.4; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (1×10^6 per vial)

Stability: 15 passages

Application: Binding assay or use as immunogen

Freeze Medium: 95% complete growth medium, 5% DMSO

Complete Growth Medium: F12K, 10% FBS

Culture Medium: F12K, 10% FBS, 8 μ g/ml Puromycin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

CTLA4 or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152 (cluster of differentiation 152), is a protein receptor that, functioning as an immune checkpoint, downregulates immune responses. CTLA4 is constitutively expressed in Tregs but only upregulated in conventional T cells after activation. It acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritidis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

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REPRESENTATIVE DATA

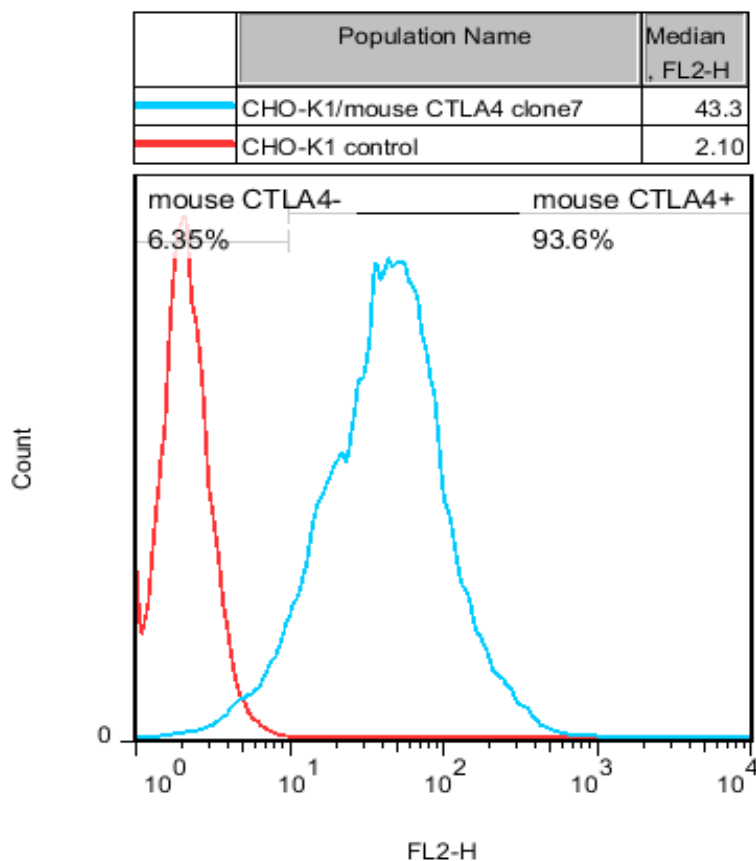


Figure 1. FACS analysis of mouse CTLA4 expression in CHO-K1/mouse CTLA4 cells.

III. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO₂.
7. Add antibiotic the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

IV. REFERENCES

1. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, Golstein P (1987). "A new member of the immunoglobulin superfamily--CTLA-4". *Nature*. 328 (6127): 267–70.
2. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, Thompson CB, Bluestone JA (August 1994). "CTLA-4 can function as a negative regulator of T cell activation". *Immunity*. 1 (5): 405–13.

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