

Human Recombinant ICOS Stable Cell Line
Cat. No. M00649

Version 08292017

I. INTRODUCTION

Catalog Number: M00649

Cell Line Name: CHO-K1/ICOS

Gene Synonyms: AILIM; CD278; CVID1

Expressed Gene: Codon Optimized from NM_012092.3; 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

The protein encoded by this gene belongs to the CD28 and CTLA-4 cell-surface receptor family. ICOS (Inducible T-cell COStimulator) is expressed on activated T cells. It forms homodimers and plays an important role in cell-cell signaling, immune responses, and regulation of cell proliferation. It is thought to be important for Th2 cells in particular.

§: 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.

III. REPRESENTATIVE DATA

Protein Expression Validation

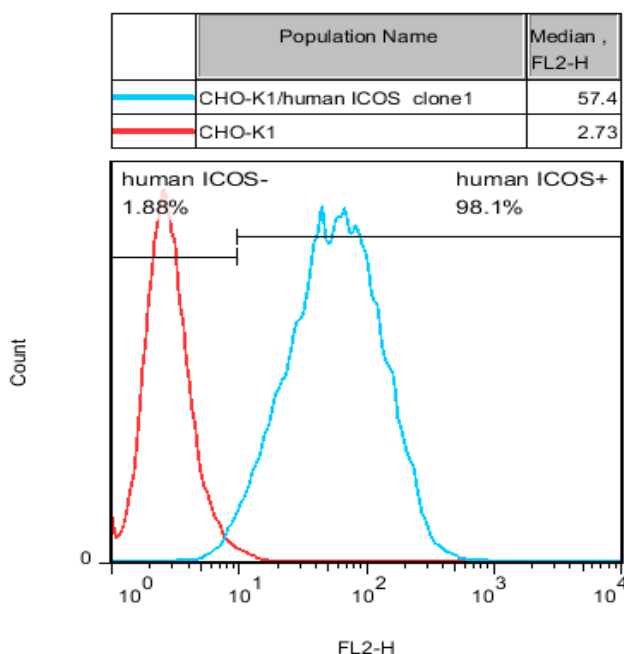


Figure 1. FACS analysis of ICOS expression in CHO-K1 cells.

IV. 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 minutes and remove the medium.
4. Re-suspend 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 an incubator at 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 0.25% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25200-072) 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 to detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.

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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 5 minutes and remove the medium.
6. Re-suspend the cells in culture medium and add the cell suspension to a new culture dish.
7. Grow the cells in an incubator at 37°C, 5% CO₂.

Subcultivation Ratio: 1:4 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Metzger TC1, et.al. ICOS Promotes the Function of CD4+ Effector T Cells during Anti-OX40-Mediated Tumor Rejection [J]. Cancer Res., 2016, 76 (13): 3684-3689.
2. Yoshinaga SK, et.al. T-cell co-stimulation through B7RP-1 and ICOS [J]. Nature, 1999, 402 (6763): 827–832.
3. Rudd CE, Schneider H. Unifying concepts in CD28, ICOS and CTLA4 co-receptor signaling [J]. Nature Reviews. Immunology, 2003, 3 (7): 544-556.
4. Dong C, et.al. ICOS co-stimulatory receptor is essential for T-cell activation and function [J]. Nature, 2001, 409 (6816): 97-101.

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