

Human Recombinant Tim3 Stable Cell Line**Cat. No. M00531****Version 04282015**

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I. INTRODUCTION

Catalog Number: M00531

Cell Line Name: CHO-K1/Tim3

Gene Synonyms: HAVCR2 ; CD366; HAVcr-2; KIM-3; TIM3; TIMD-3; TIMD3

Expressed Gene: Codon Optimized from NM_032782.4; 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

T cell immunoglobulin mucin-3 (TIM-3) is a member of the T-cell Immunoglobulin- and Mucin-domain-containing family of type I membrane glycoproteins that regulate autoimmune and allergic disease. TIM-3 is selectively expressed on Th1 cells and interacts with galectin-9. It negatively regulates Th1 responses and affects macrophage activation. The 280 amino acid mature human TIM-3 contains a V-type Ig-like domain that shows multiple polymorphisms, followed by a mucin-like domain in the 171 amino acid extracellular region, which shares 60% amino acid identity with mouse TIM-3 ECD.

§: 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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III. REPRESENTATIVE DATA

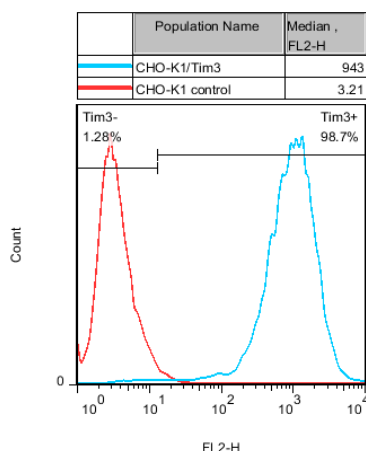


Figure 1. FACS analysis of Tim3 expression in CHO-K1/Tim3 cells.

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 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

* GenScript uses a PCR-based method to test the mycoplasma. Our PCR mycoplasma test covers 11 of the most common species of mycoplasma (M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae, M. capricolum) and one species of Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

V. REFERENCES

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2. Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA, Freeman GJ, Kuchroo VK (Feb 2002). Nature 415 (6871): 536–41.

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