Human Recombinant TIGIT Stable Cell Line
Cat. No. M00542 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00542
Cell Line Name: CHO-K1/TIGIT
Gene Synonyms: WUCAM, Vstm3
Expressed Gene: Codon Optimized from NM_173799.3; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (1×106 per vial)
Stability: 15 passages
Application: Binding assays or use as immunogen
Freeze Medium: 95% complete growth medium, 5% DMSO
Complete Growth Medium: F12K, 10% FBS
Culture Medium: F12K, 10% FBS, 6 μg/ml Puromycin
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

TIGIT (T cell immunoreceptor with Ig and ITIM domains) was recently characterized as an inhibitory receptor, which is expressed mainly on NK, Treg, CD8+ T, and CD4+ T cells. TIGIT harbors an immunoglobulin tail tyrosine (ITT)-like phosphorylation motif and an ITIM (immunoreceptor tyrosine-based inhibition motif) in its cytoplasmic tail. The poliovirus receptor (PVR or CD155) was identified as the physical ligand of TIGIT with high affinity, and PVRL2 (Nectin2 or CD112) also binds to TIGIT with a weaker binding capacity. TIGIT/PVR engagement suppresses T cell activation through IL-10 secretion mediated by dendritic cells. Furthermore, TIGIT also exerts an intrinsic inhibitory function to T cell activation.

§: 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.

REPRESENTATIVE DATA

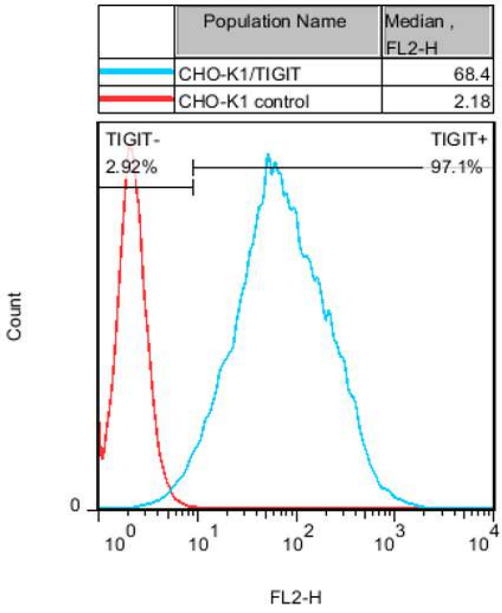

Figure 1. FACS analysis of TIGIT expression in CHO-K1/TIGIT cells.

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 %CO_2.
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 5 min, 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 %CO_2.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

IV. REFERENCES

1. Mahoney KM1, Rennert PD2, Freeman GJ3. Combination cancer immunotherapy and new immunomodulatory targets. Nat Rev Drug Discov. 2015 Jul 31; 14(8):561-84.
2. Man Li, Pengyan Xia. T-cell Immunoglobulin and ITIM Domain (TIGIT) Receptor/Poliovirus Receptor (PVR) Ligand Engagement Suppresses Interferon-γ Production of Natural Killer Cells via β-Arrestin 2-mediated Negative Signaling. J Biol Chem. 2014 Jun 20; 289(25): 17647–17657.

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