

Mouse Recombinant TIGIT Stable Cell Line
Cat. No. M00585**Version 11122018**

I. INTRODUCTION

Catalog Number: M00585

Cell Line Name: CHO-K1/mouse TIGIT

Gene Synonyms: VSIG9, VSTM3

Expressed Gene: Codon Optimized from NM_001146325.1; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells ($>1 \times 10^6$ cells/vial)

Stability: 15 passages

Application: Binding assay or use as immunogen

Freeze Medium: 95% Culture Medium, 5% (V/V) DMSO

Complete Growth Medium: F12K, 10% FBS

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

Mycoplasma Status: Not detected*

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

TIGIT (also called T cell immunoreceptor with Ig and ITIM domains) is one newly discovered immune receptor on some percentage of T cells and Natural Killer Cells (NK). It is also identified as WUCAM and Vstm3. TIGIT could bind to CD155 (PVR) on dendritic cells (DCs), macrophages, etc. with high affinity, and also to CD112 (PVRL2) with lower affinity. Research has shown that TIGIT-Fc fusion protein could interact with PVR on dendritic cells and increase its IL-10 secretion level/decrease its IL-12 secretion level under LPS stimulation, and also inhibit T cell activation in vivo. TIGIT's inhibition of NK cytotoxicity can be blocked by antibodies against its interaction with PVR and the activity is directed through its ITIM domain

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

Protein Expression Validation

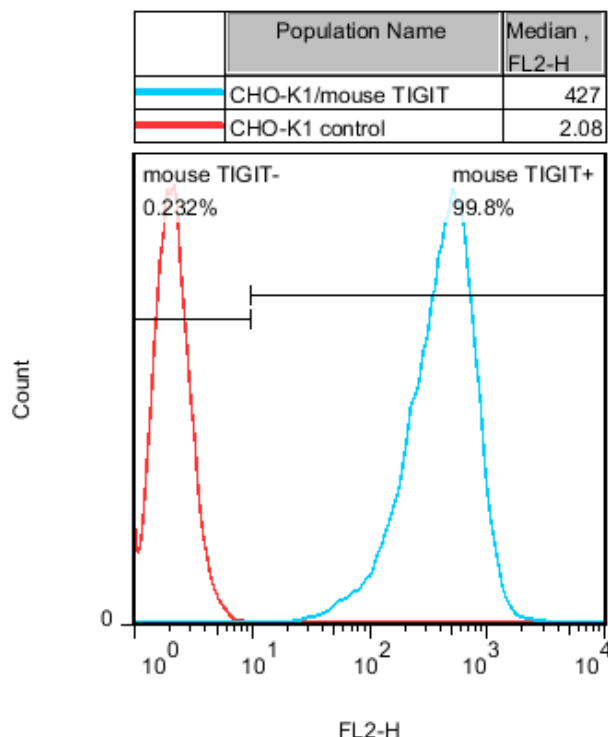


Figure 1. FACS analysis of mouse TIGIT expression in CHO-K1/mouse TIGIT cells.

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 800 rpm for 4 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 0.25% (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 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 800 rpm for 4 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% CO₂.

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

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Yu X, Harden K, Gonzalez LC, Francesco M, Chiang E, Irving B, Tom I, Ivelja S, Refino CJ, Clark H, Eaton D, Grogan JL (Jan 2009). "The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells". *Nat Immunol.* 10 (1): 48–57.
2. Boles KS, Vermi W, Facchetti F, Fuchs A, Wilson TJ, Diacovo TG, Cella M, Colonna M (Mar 2009). "A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC". *European Journal of Immunology.* 39 (3): 695–703.
3. Levin SD, Taft DW, Brandt CS, and 21 others (Apr 2011). "Vstm3 is a member of the CD28 family and an important modulator of T-cell function". *European Journal of Immunology.* 41 (4): 902–15.
4. Stanietsky N, Simic H, Arapovic J, Toporik A, Levy O, Novik A, Levine Z, Beiman M, Dassa L, Achdout H, Stern-Ginossar N, Tsukerman P, Jonjic S, Mandelboim O (Oct 2009). "The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity". *Proc Natl Acad Sci U S A.* 106 (42): 17858–63

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