Human Recombinant GITR Stable Cell Line
Cat. No. M00539 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00539

Cell Line Name: CHO-K1/GITR

Gene Synonyms: TNFRSF18; AITR; CD357; GITR-D

Expressed Gene: Codon Optimized from NM_004195.2; 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, 6 μg/ml Puromycin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

Tumor necrosis factor receptor superfamily member 18 (TNFRSF18) also known as activation-inducible TNFR family receptor (AITR) or glucocorticoid-induced TNFR-related protein (GITR) is a protein that in humans is encoded by the TNFRSF18 gene. GITR is currently of interest to immunologists as a co-stimulatory immune checkpoint molecule.

§: 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.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

III. REPRESENTATIVE DATA

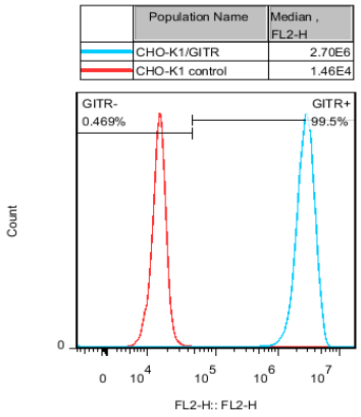

Figure 1. FACS analysis of GITR expression in CHO-K1/GITR cells.

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 %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 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.

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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

* 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. Nocentini G, Giunchi L, Ronchetti S, Krausz LT, Bartoli A, Moraca R, Migliorati G, Riccardi C (Jul 1997). "A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis". Proc Natl Acad Sci U S A 94 (12): 6216–21.

GenScript USA Inc,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: http://www.genscript.com

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