Human Recombinant GITR Stable Cell Line
Cat. No. M00607 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00607
Cell Line Name: GS-H2/GITR
Gene Synonyms: TNFRSF18, AITR, CD357, GITR, GITR-D
Expressed Gene: Codon Optimized from NM_004195.2
Host Cell: GS-H2
Quantity: Two vials of frozen cells (1×10^6 per vial)
Stability: 20 passages
Application: *in vitro* functional assay
Freeze Medium: 95% complete growth medium, 5% DMSO
Complete Growth Medium: MEM, 10% FBS
Culture Medium: MEM, 10% FBS, 2 μg/ml Puromycin, 200 μg/ml Hygromycin B
Mycoplasma160: Negative
Functional Performance: For GITRL, Signal / Background (S/B) > 3
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

GITR (glucocorticoid-induced TNFR-related protein) was identified as a new member of the TNF receptor superfamily. GITR is currently of interest to immunologists as a co-stimulatory immune checkpoint molecule. This receptor has been shown to have increased expression upon T-cell activation, and it is thought to play a key role in dominant immunological self-tolerance maintained by CD25+/CD4+ regulatory T cells. The modulation of GITR is listed as one of the top 25 most promising research areas by the NCI, and has demonstrated potential in both antitumor and vaccine settings.

III. REPRESENTATIVE DATA

- Protein Expression Validation

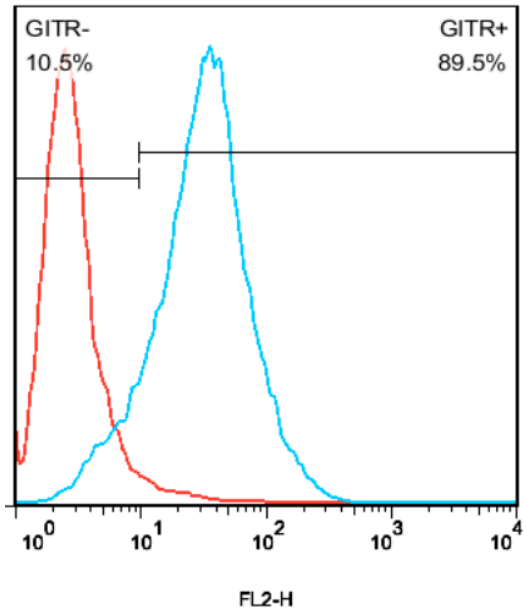

Figure 1. Flow cytometry analysis of GITR protein expression in GS-H2/GITR cells. Red: GS-H2, Blue: GS-H2/GITR.

- Functional Validation by *in vitro* Assay

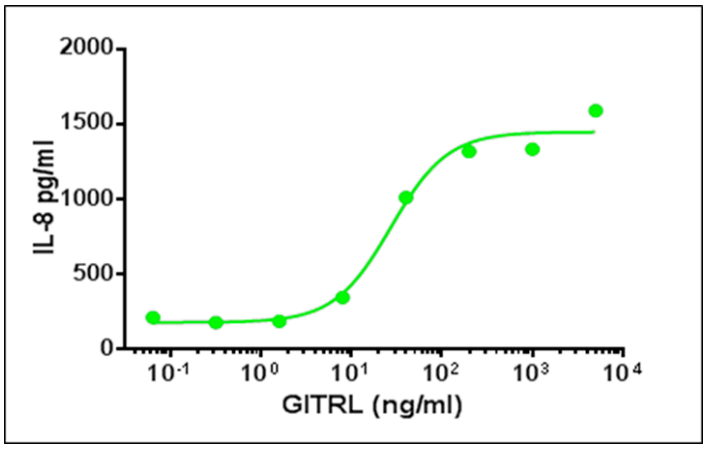

Figure 2. Functional evaluation of GS-H2/GITR cells by measuring GITRL induced IL-8 production.

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 in the following day.

Sub-culturing Protocol

1. Centrifuge the cells at 200 x g for 5min, and remove the medium.
2. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
3. Grow the cells in incubator with 37°C, 5 % CO_2.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Gurney AL, Marsters SA, Huang RM, et al. (1999). "Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR". Curr. Biol. 9 (4): 215–8.

2. Zhang Z, Henzel WJ (2005). "Signal peptide prediction based on analysis of experimentally verified cleavage sites". Protein Sci. 13 (10): 2819–24.

3. Schaer DA, Cohen AD, Wolchok JD. Anti-GITR antibodies--potential clinical applications for tumor immunotherapy. Curr Opin Investig Drugs. 2010 Dec;11(12):1378-86.

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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