Human Recombinant 4-1BB Stable Cell Line

Cat. No. M00609 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00609
Cell Line Name: GS-H2/4-1BB
Gene Synonyms: 4-1BB; CD137; ILA; TNFRSF9
Expressed Gene: Codon Optimized from NM_001561.5; no expressed tags
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, 200 μg/ml Hygromycin B, 2 μg/ml Puromycin
Mycoplasma Status: Negative
Functional Performance: For 4-1BBL (ligand of 4-1BB), Signal / Background (S/B) > 3
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

4-1BB is a member of the tumor necrosis factor (TNF) receptor family. Its alternative names are tumor necrosis factor receptor superfamily member 9 (TNFRSF9), CD137 and induced by lymphocyte activation (ILA). It is currently of interest to immunologists as a co-stimulatory immune checkpoint molecule.

4-1BB can be expressed by activated T cells, but to a larger extent on CD8 than on CD4 T cells. In addition, CD137 expression is found on dendritic cells, follicular dendritic cells, natural killer cells, granulocytes and cells of blood vessel walls at sites of inflammation.

The best characterized activity of 4-1BB is its costimulatory activity for activated T cells. Crosslinking of 4-1BB enhances T cell proliferation, IL-2 secretion, survival and cytolytic activity. Further, it can enhance immune activity to eliminate tumors in mice.

III. REPRESENTATIVE DATA

- Protein Expression Validation

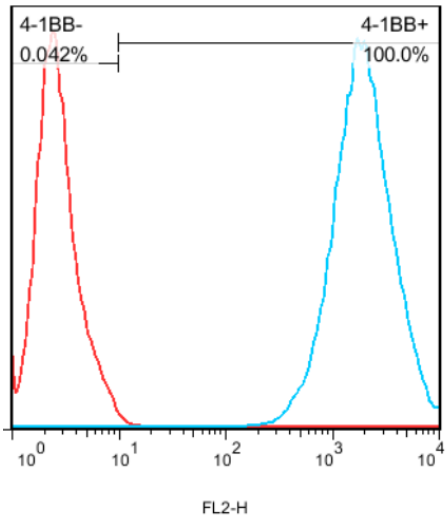

Figure 1. Flow cytometry analysis of 4-1BB protein expression in GS-H2/4-1BB cells. Red: GS-H2, Blue: GS-H2/4-1BB.

- Validation by *in vitro* Functional Assay

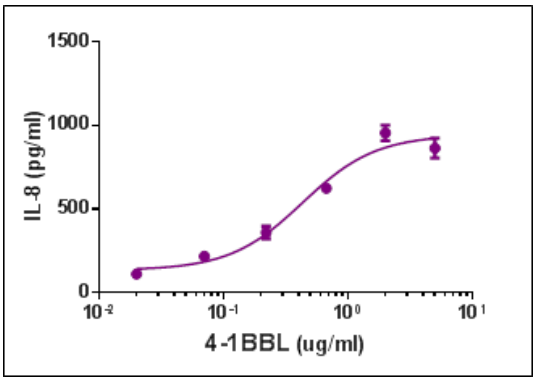

Figure 2. Functional analysis of 4-1BBL on GS-H2/4-1BB cells by measuring IL-8 release.

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. Kwon BS, Weissman SM (1989). "cDNA sequences of two inducible T-cell genes". Proc. Natl. Acad. Sci. U.S.A. 86 (6): 1963–7.
2. Schwarz H, Tuckwell J, Lotz M (1993). "A receptor induced by lymphocyte activation (ILA): a new member of the human nerve-growth-factor/tumor-necrosis-factor receptor family". Gene. 134 (2): 295–8.
3. Sica G, Chen L (2000). "Biochemical and immunological characteristics of 4-1BB (CD137) receptor and ligand and potential applications in cancer therapy". Arch. Immunol. Ther. Exp. (Warsz.). 47 (5): 275–9.
4. Schwarz H (2005). "Biological activities of reverse signal transduction through CD137 ligand". J. Leukoc. Biol. 77 (3): 281–6

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