Cyno Recombinant 4-1BB Stable Cell Line
Cat. No. M00569 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00569
Cell Line Name: CHO-K1/cyno 4-1BB
Gene Synonyms: ILA; 4-1BB; CD137; CDw137, TNFRSF9
Expressed Gene: Codon Optimized from XM_005544888.2; 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, 8 μg/ml Puromycin
Mycoplasma Status: Negative
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, 4-1BB expression is found on dendritic cells, follicular dendritic cells, natural killer cells, granulocytes and cells of blood vessel walls at sites of inflammation.

§: 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

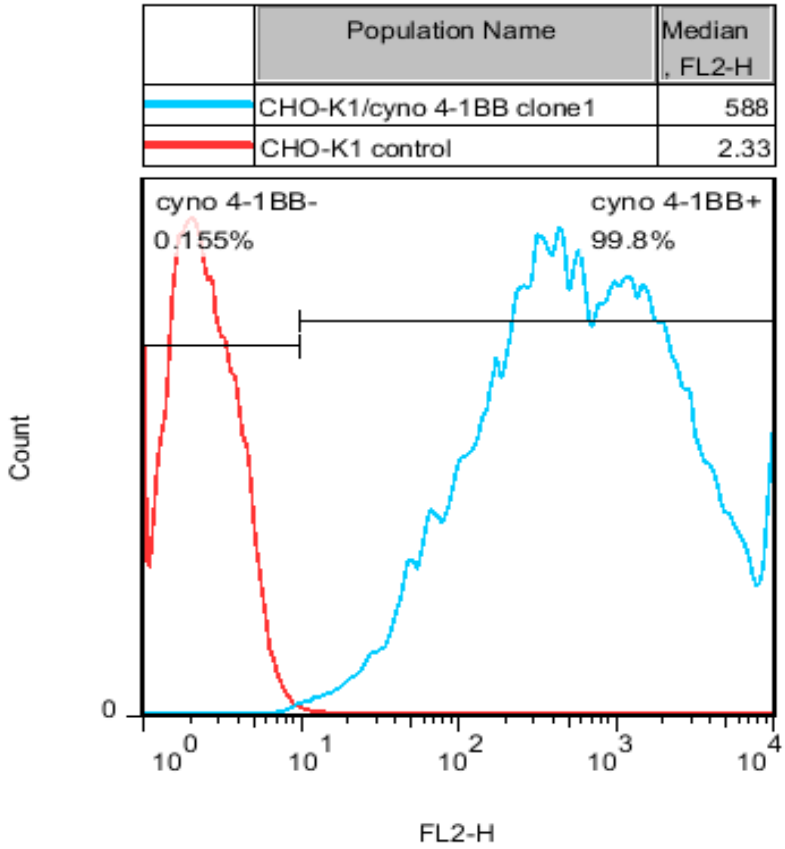

Figure 1. FACS analysis of cyno 4-1BB expression in CHO-K1/cyno 4-1BB cells.

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 5min, 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. Jang IK, Lee ZH, Kim YJ, Kim SH, Kwon BS (Jan 1998). "Human 4-1BB (CD137) signals are mediated by TRAF2 and activate nuclear factor-kappa B". Biochem. Biophys. Res. Commun. 242 (3): 613–20.
2. *Arch RH, Thompson CB (Jan 1998). "4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor kappaB"*. Mol. Cell. Biol. 18 (1): 558–65

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