**Human Recombinant B7-H4 Stable Cell Line**
**Cat. No. M00537** **Version 04282015**



## I. INTRODUCTION

Catalog Number: M00537
Cell Line Name: CHO-K1/B7-H4
Gene Synonyms: VTCN1 ; B7H4; B7S1; B7X; B7h.5; PRO1291; VCTN1
Expressed Gene: Codon Optimized from NM_024626.3; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells ($1\times10^6$ 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, 600 μg/ml G418
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon receipt

## II. BACKGROUND

B7-H4 is a transmembrane protein that binds an unknown receptor on activated T cells resulting in inhibition of T-cell effector function via cell cycle arrest, decreased proliferation, and reduced IL-2 production. B7-H4 is up-regulated on the surface of cancer cells and immunosuppressive tumor-associated macrophages (TAMs) in a variety of human cancers.

*§: 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.*


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## III. REPRESENTATIVE DATA

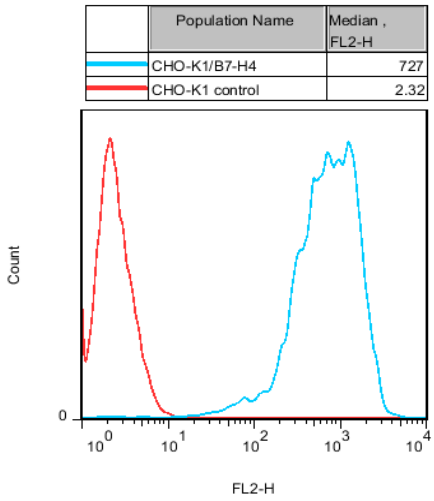


**Figure 1.** FACS analysis of B7-H4 expression in CHO-K1/B7-H4 cells.

## 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.


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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

* 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

1. Mahoney KM1, Rennert PD2, Freeman GJ3. Combination cancer immunotherapy and new immunomodulatory targets.Nat Rev Drug Discov. 2015 Jul 31;14(8):561-84.
2. Jenessa B. Smith, Caitlin Stashwick, and Daniel J. Powell, Jr.b. B7-H4 as a potential target for immunotherapy for gynecologic cancers: A closer look. Gynecol Oncol. 2014 Jul; 134(1): 181–189.

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