Human Recombinant CD80 Stable Cell Line

Cat. No. M00545 **Version 01152018**

I. INTRODUCTION

Catalog Number: M00545
Cell Line Name: CHO-K1/CD80
Gene Synonyms: B7; B7-1; B7.1; BB1; CD28LG; CD28LG1; LAB7
Expressed Gene: Codon Optimized from NM_005191.3; 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

Cluster of Differentiation 80 (also CD80 and B7-1) is a protein found on activated B cells and monocytes that provides a costimulatory signal necessary for T cell activation and survival. It is the ligand for two different proteins on the T cell surface: CD28 (for autoregulation and intercellular association) and CTLA-4 (for attenuation of regulation and cellular disassociation). CD80 works in tandem with CD86 to prime T cells

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 160 of the most common strains of mycoplasma covering approximately 95% of mycoplasma contaminations (A. laidlawii,M.arginini, M. fermentans, M. hominis M. hyorhinis, and M. orale. Furthermore, the primers also detect at least another 12 Acholeplasma species, 110 Mycoplasma species, 33 Spiroplasma species, and 3 Ureaplasma species.), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Protein Expression Validation

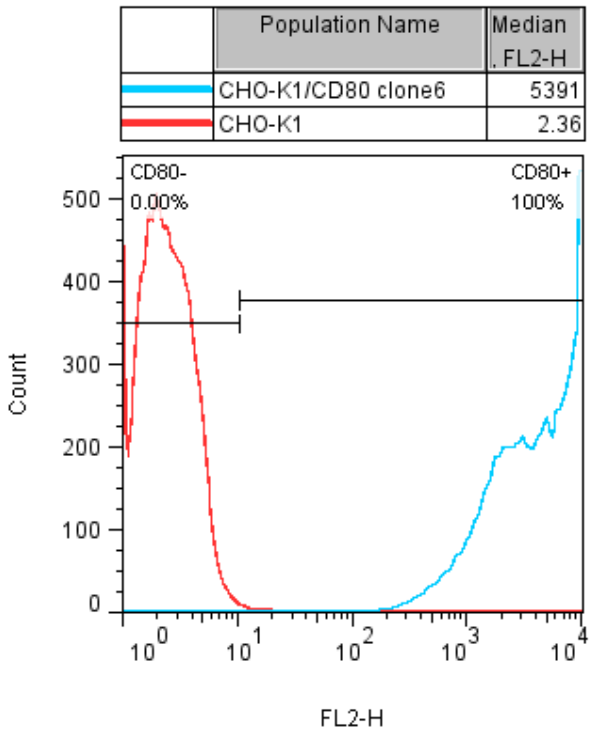

Figure 1. FACS analysis of CD80 expression in CHO-K1/CD80 cells.

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 0.25% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25200-072) 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 5 min, 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: 1:4 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

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. Peach, R J; Bajorath J; Naemura J; Leytze G; Greene J; Aruffo A; Linsley P S (Sep 1995). "Both extracellular immunoglobin-like domains of CD80 contain residues critical for binding T cell surface receptors CTLA-4 and CD28". J. Biol. Chem. (UNITED STATES) 270 (36): 21181–7.

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