Human Recombinant CD80 Stable Cell Line

Cat. No. M00614 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00614
Cell Line Name: GS-C1/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: GS-C1
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: F12K, 10% FBS
Culture Medium: F12K, 10% FBS, 4 μg/ml Puromycin
Mycoplasma Status: Negative
Functional Performance: For Ipilimumab, Signal / Background (S/B) > 3
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.

III. REPRESENTATIVE DATA

- Protein Expression Validation

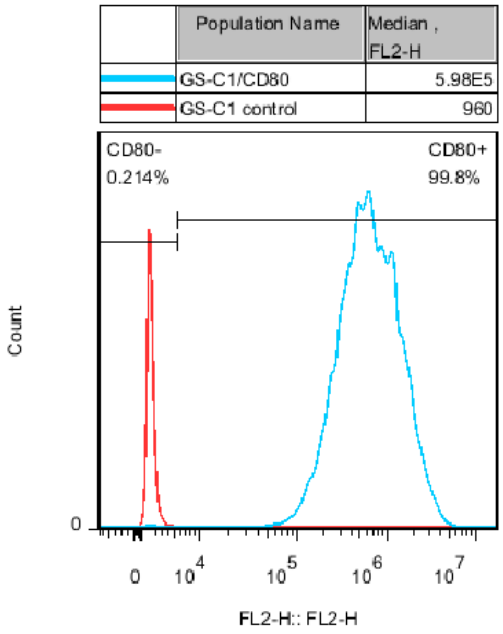

Figure 1. FACS analysis of CD80 expression in GS-C1/CD80 cells.

- Validation by *in vitro* Functional Assay

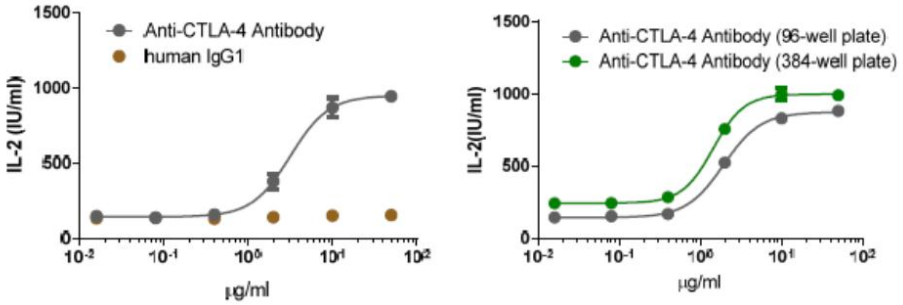

Figure 2. Functional evaluation of GS-C1/CD80 by cell-based anti-CTLA4 activity assay. The EC50 curves studies with Ipilimumab (Yervoy®) were shown in 96 and 384-well plate formats, respectively. Human IgG1 was used as a negative controls. GS-J1 (CD28 expressed T cells, M00611) were co-cultured with GS-C1/CD80 together with CTLA-4 fusion protein and Ipilimumab.

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 force 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. 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 force 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 is recommended

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.

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

For Research Use Only.

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 120 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.