Cyno Recombinant PD-L2 Stable Cell Line

Cat. No. M00629 **Version 08112017**

I. INTRODUCTION

Catalog Number: M00629
Cell Line Name: CHO-K1/cyno PD-L2
Gene Synonyms: Btdc; B7-DC ; PDCD1LG2
Expressed Gene: Codon Optimized from XM_005581781.2; 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

Programmed cell death 1 ligand 2 (PD-L2) is a protein that is encoded by the PDCD1LG2 gene in humans. PDCD1LG2 has also been designated as CD273 (cluster of differentiation 273). Inhibitory molecules of the B7/CD28 family play a key role in the induction of immune tolerance in the tumor microenvironment. The programmed death-1 receptor (PD-1), with its ligands PD-L1 and PD-L2, constitutes an important member of these inhibitory pathways. PD-L2 expression was initially thought to be restricted to antigen-presenting cells such as macrophages and dendritic cells (DCs). However, PD-L2 expression can be induced on a wide variety of other immune cells and nonimmune cells depending on micro environmental stimuli.

§: 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.

III. REPRESENTATIVE DATA

Protein Expression Validation

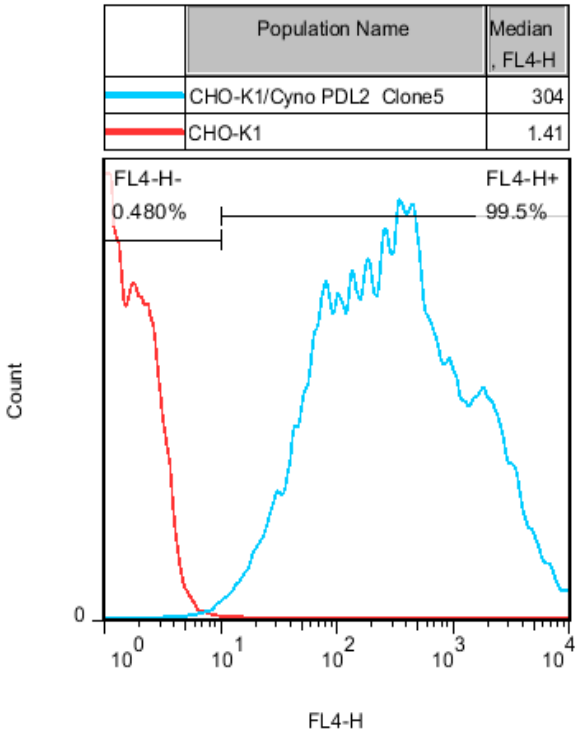

Figure 1. FACS analysis of cyno PD-L2 expression in CHO-K1 cells.

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 minutes and remove the medium.
4. Re-suspend 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 an incubator at 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 to 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 minutes and remove the medium.
6. Re-suspend the cells in culture medium and add the cell suspension to a new culture dish.
7. Grow the cells in an incubator at 37°C, 5% CO_2.

Subcultivation Ratio: 1:4 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Y. Latchman, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation [J]. Nature Immunology, 2001, 2(3): 261–268.
2. Esdy N. Rozali, et al., Programmed Death Ligand 2 in Cancer-Induced Immune Suppression [J]. Clinical and Developmental Immunology, 2012, 2012:656340.
3. W. J. Lesterhuis, H. Steer, and R. A. Lake, PD-L2 is predominantly expressed by Th2 cells [J]. Molecular Immunology, 2011, 49(1-2): 1–3.
4. N. Messal, et al. PD-L2 is expressed on activated human T cells and regulates their function [J]. Molecular Immunology, 2011, 48(15-16): 2214–2219.

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