

**Mouse Recombinant PD-L1 Stable Cell Line**  
**Cat. No. M00567****Version 04282015****I. INTRODUCTION**

Catalog Number: M00567

Cell Line Name: CHO-K1/mouse PD-L1

Gene Synonyms: CD274 ; B7-H; B7H1; PDCD1L1; PDCD1LG1; PDL1

Expressed Gene: Codon Optimized from NM\_021893.3; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells ( $1 \times 10^6$  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, 600 µg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon receipt

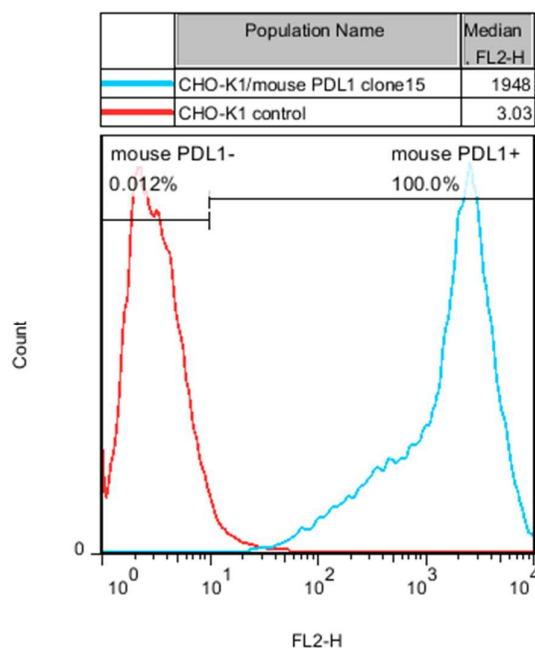
**II. BACKGROUND**

Programmed death-ligand 1 (PD-L1) also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1) is a protein that in humans is encoded by the CD274 gene. The formation of PD-1 receptor / PD-L1 or B7.1 receptor /PD-L1 ligand complex transmits an inhibitory signal which reduces the proliferation of these CD8+ T cells at the lymph nodes. After that PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis, which is further mediated by a downregulation of the Bcl-2 gene.

§: 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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## REPRESENTATIVE DATA



**Figure 1.** FACS analysis of mouse PD-L1 expression in CHO-K1/mouse PD-L1 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<sub>2</sub>.
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 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<sub>2</sub>.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

**IV. 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. Butte MJ, Peña-Cruz V, Kim MJ, Freeman GJ, Sharpe AH (August 2008). "Interaction of human PD-L1 and B7-1". Mol Immunol. 45 (13): 3567–72.

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