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Cynomolgus Recombinant PD-L2 Stable Cell Line
Cat. No. M00629

I. INTRODUCTION

Recombinant CHO-K1 cells stably overexpress *Macaca fascicularis* programmed cell death 1 ligand 2 (PD-L2) on the surface. The surface expression of cyno PD-L2 is validated by FACS analysis. This cell line is recommended for cell-based binding assay to screen antibodies against PD-L2 or to measure binding affinity between PD-L2 and anti-PD-L2 antibodies.

Catalog Number: M00629
Cell Line Name: CHO-K1/cyno PD-L2
Gene Synonyms: B7DC, Btdc, CD273, PDCD1LG2, PDCD1L2, PDL2
Expressed Gene: NCBI reference sequence XM_005581781.2; no expressed tags
Target Protein: XP_005581838.1
Host Cell: CHO-K1
Size: Two vials of frozen cells (>1×10^6 per vial in 1 mL)
Culture Properties: Adherent
Freeze Medium: 95% complete growth medium, 5% (V/V) DMSO (Cat. No. D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. No. 21127-022, Life Technologies), 10% FBS (Cat. No. 10099-141, Life Technologies)
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 8 μg/ml Puromycin (Cat. No. A11138-03, Life Technologies)
Stability: Stable through more than 16 passages without significant changes in assay performance or expression profile.
Application: Binding assay or use as immunogen
Mycoplasma Status: Negative. The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit (Cat. No. LT07-318, Lonza).
Storage: Store cells in liquid nitrogen immediately upon receipt. Thaw and recover cells within

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one year from the date received.

II. BACKGROUND

Programmed cell death 1 ligand 2 (PD-L2) is a protein that in humans is encoded by the PDCD1LG2 gene. 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). PD-L2 expression can be induced on a wide variety of other immune cells and nonimmune cells depending on microenvironmental stimuli.

III. REPRESENTATIVE DATA

FACS Analysis

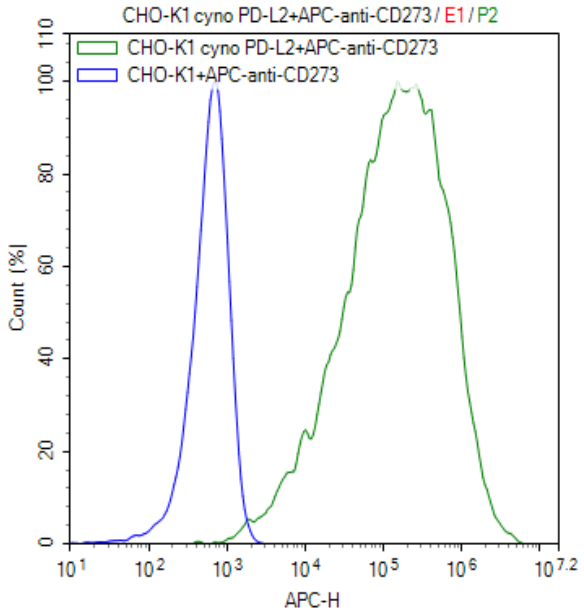

Figure 1. FACS analysis of cell surface expression of cyno PD-L2 on CHO-K1/cyno PD-L2 cells. The CHO-K1/cyno PD-L2 cells (green) and the negative control CHO-K1 cells (blue) were probed using APC-conjugated anti-human CD273 (Cat. No. 345507, Biolegend).

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial containing the frozen cells from liquid nitrogen tank and place into a 37°C water bath immediately.

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2. Thaw the cells quickly (within 1-2 minutes) by gently swirling the vial. Do not vortex the cells.
3. When the cells are almost completely thawed, take the vial out of the water bath and decontaminate it with 70% ethanol.
4. In a biosafety hood, transfer the cells to a sterile 15 ml conical tube. Add 9 ml of complete growth medium to the cells.
5. Pellet cells by centrifugation at 200 × g for 3-5 minutes at room temperature.
6. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure that the cell pellet is not disturbed.
7. Resuspend the cells by gently flicking the tube. Gently add in 10 ml of complete growth medium.
8. Transfer the cell suspension into a 10 cm culture dish containing 10 ml of complete growth medium.
9. Grow the cells in an incubator at 37°C with 5% CO_2.
10. The cells will attach the dish in about 2-4 days. Check the status of the cells every day and don't disturb the cells till most cells attach well.
11. Change the medium with culture medium when cells grow well.

Sub-culturing Protocol

1. Remove the culture medium from the cells.
2. Wash cells with sterile PBS to remove all traces of serum which contains trypsin inhibitors.
3. Add 0.25% Trypsin/EDTA (Cat. No. 25200, Gibco) solution to the culture dish and observe the cells under an inverted microscope until the cell layer has dispersed (usually within 3-5 minutes).
   Notes: 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, place the dish in a 37°C incubator for about 2 minutes.
4. Add 6-8 ml of complete growth medium to the culture dish, aspirate the medium with cells by gentle pipetting and then add into a sterile falcon tube.
5. Centrifuge the cells at 200 x g for 5 minutes, and remove the medium.
6. Resuspend 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 with 5% CO_2.
8. The cells will attach the dish in about 4 days. Don't disturb the cells till most cells attach well.
9. Change the medium with culture medium when cells grow well.
   Notes:
   Subcultivation Ratio: 1:3 to 1:8.
   Medium Renewal: Every 2 to 3 days.

Cryopreservation Protocol

1. Remove the cell culture medium, wash the cells with PBS once (optional), gently add enough trypsin to cover the cells and incubate for approximately 2 minutes in a 37°C incubator.
2. Resuspend in cell culture medium and transfer into a sterile 50 ml conical tube.
3. Count the viable cells using a hemocytometer. If preferred, also determine the cell viability. Cell

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viability should be at least 90% for good cryopreservation.

4. Centrifuge the cells at about 200 × g for 5 minutes at room temperature to pellet cells. Remove the supernatant gently without disturbing the cell pellet.
5. Resuspend cells by adding freezing medium to the tube to the required cell density (2-5 × 10^6 cells/ml for best results).
6. Aliquot 1 ml each into cryogenic storage vials and secure the lids.
7. Transfer the vials into a cryo-freezing container at room temperature and put into a -80°C freezer. The temperature inside the cryo-freezing container should decrease steadily by 1°C/minute.
8. After approximately 24 hours, remove the vials from the cyro-freezing container and transfer into liquid nitrogen for long term storage.

V. REFERENCES

1. Y. Latchman, *et al*. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nature Immunology*, 2001, 2 (3): 261–268.
2. Esdy N. Rozali, *et al*., Programmed Death Ligand 2 in Cancer-Induced Immune Suppression. *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. *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. *Molecular Immunology*, 2011, 48 (15-16): 2214–2219.

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