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Data Sheet

PD-L2 / TCR activator - CHO Recombinant Cell Line
Catalog #79632

Product Description
Recombinant CHO-K1 cells constitutively expressing human PD-L2 (Programmed Cell Death 1 Ligand 2 or CD273, GenBank accession #NM_025239) and an engineered T-cell receptor (TCR) activator.

Background
The binding of Programmed Cell Death Protein 1 (PD-1), a receptor expressed on activated T-cells, to its ligands, PD-L1 and PD-L2, negatively regulates immune responses. PD-1 ligands are found on most cancers, and the PD-1:PD-L1/2 interaction inhibits T-cell activity and allows cancer cells to escape immune surveillance. The PD-1:PD-L1/2 pathway is also involved in regulating autoimmune responses, making these proteins promising therapeutic targets for a number of cancers, as well as multiple sclerosis, arthritis, lupus, and type I diabetes.

Applications
- Screen for activators or inhibitors of PD-1 signaling in a cellular context
- Screen PD-L2 antibodies for binding affinity
- Characterize the biological activity of PD-1 interactions with PD-L2

Format
Each vial contains 2.5 x 10^6 cells in 1 ml of FBS with 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 3 (BPS Cat. #60186): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 3A (BPS Cat. #60188): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin and 500 µg/ml of Hygromycin B to ensure recombinant expression..

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Cells should be grown at 37˚C with 5% CO_2 using Growth Medium 3A. PD-L2 / TCR activator CHO cells should exhibit a typical cell division time of ~24 hours.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 3 **(no Geneticin or Hygromycin B)**, spin down cells, remove supernatant, and re-suspend the cells in pre-warmed Thaw Medium 3 **(no Geneticin or Hygromycin B)**. Then transfer the re-suspended cells to a T25 flask, and culture in a 37˚C CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 **(no Geneticin or Hygromycin B)**, and continue growing in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At the first passage, switch to Growth Medium 3A **(contains Geneticin and Hygromycin B)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from the culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 3A and transfer to a tube, spin down the cells, remove the supernatant, and then re-suspend the cells and seed appropriate aliquots of the cell suspension into new culture vessels. Sub cultivation ration: 1:10 to 1:20 twice a week.

To freeze down the cells, rinse the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 3 **(no Geneticin or Hygromycin B)** and count the cells, then transfer to a tube, spin down the cells, and resuspend in 4˚C Freezing Medium (10% DMSO + 90% FBS) at ~2.5 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80˚C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

Functional Validation and Assay Performance
Expression of human PD-L2 in CHO-K1 cells was confirmed by FACS.

The functionality of the cell line was validated using a PD-1:PD-L2 cell-based assay. In this assay, Jurkat T-cells expressing an NFAT reporter with constitutive expression of PD-1 (PD-1 / NFAT-Reporter Jurkat, BPS Bioscience #60535) are used as effector cells; PD-L2 / TCR activator / TCR activator CHO cells are used as target cells. When these two cells are co-cultivated, TCR complexes on the effector cells are activated by the TCR activator on the target cells, resulting in expression of the NFAT luciferase reporter. However, PD-1 and PD-L2 ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by an anti-PD-1 antibody. PD-1 neutralizing antibodies block the PD-1:PD-L2 interaction and promote T-cell activation, resulting in reactivation of the NFAT responsive luciferase reporter.

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Assay Principle

Materials Required by Not Supplied

- PD-1/NFAT reporter Jurkat cell line (BPS Bioscience #60535)
- Assay medium: Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 3A (BPS Bioscience #60188)
- Anti-PD-1 neutralizing antibody (BPS Bioscience #71120)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience #60690)
- Luminometer
- Thaw Medium 3 (BPS Bioscience #60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079) and 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Protocol

1. Harvest PD-L2 / TCR activator CHO cells from culture and seed cells at a density of 35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 3, BPS Bioscience #60186 (growth medium **without Geneticin and Hygromycin B**). Incubate cells at 37˚C in a CO_2 incubator for overnight.

2. **To test anti-PD-1 antibody,** the next day, prepare a serial dilution of anti-PD-1 antibody in Thaw Medium 2, BPS Bioscience #60184 (Assay medium). The concentration of antibody should be 2x the final treatment concentration of antibody. Harvest the PD-1 / NFAT-reporter Jurkat cells by centrifugation and resuspend in assay medium. Dilute the cells to $7x10^5$ cells/ml in assay medium.

   Preincubate the PD-1 / NFAT-reporter Jurkat cells (7×10^5 cells/ml) with diluted anti-PD-1 antibody (1:1 in volume) for 30 minutes. After incubation, remove the medium from PD-L2 / TCR activator CHO cells and add 100 µl of PD-1 / NFAT reporter Jurkat cells / anti-PD-1 antibody mixture to the wells. (Note: *Mix the PD-1 / NFAT-Reporter Jurkat cells with antibody well before adding to the PD-L2 / TCR activator CHOPD-L2 / TCR activator CHO cells.)*

   Final cell density of PD-1 / NFAT-Reporter Jurkat cells is 35,000 cells per well. Set up each treatment in at least triplicate.

   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

   Incubate the plates at 37˚C in a CO_2 incubator for 5 to 6 hours.

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3. After a ~5-6 hour incubation, perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of ONE-Step luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.

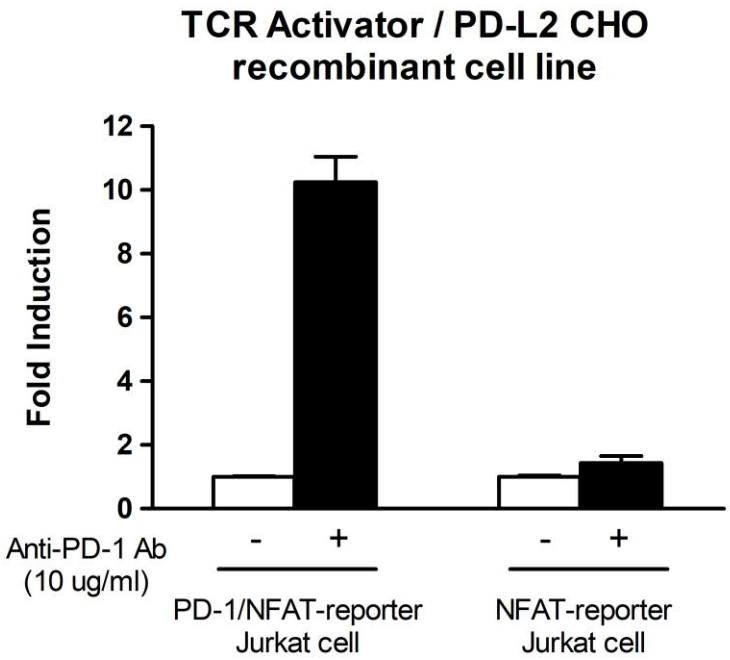
4. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

   The fold induction of NFAT luciferase reporter expression = background-subtracted luminescence of treated well / average background-subtracted luminescence of untreated control wells.

Figure 1. Cell Characterization Using a PD-1 Neutralizing Antibody.
CHO cells were seeded in a 96-well plate. The next day, PD-L2 / TCR activator CHOPD-L2 / TCR activator CHO cells were incubated with anti-PD-1 neutralizing antibody (BPS Bioscience #71120) and PD-1 / NFAT Reporter Jurkat cells (BPS Bioscience #60535) or control NFAT Reporter Jurkat cells (BPS Bioscience #60621). After incubation, ONE-Step Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure NFAT activity.

The fold induction is equal to background-subtracted luminescence of antibody-treated well / background-subtracted luminescence of untreated-control wells of each respective cell line.

A. Anti-PD-1 neutralizing antibody induced NFAT luciferase reporter activity in PD-1 / NFAT-Reporter Jurkat cells, but not control NFAT-Reporter Jurkat cells, co-cultured with PD-L2 / TCR activator CHO cells.



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B. Dose response of anti-PD-1 neutralizing antibody in PD-L2 / NFAT-Reporter Jurkat cells

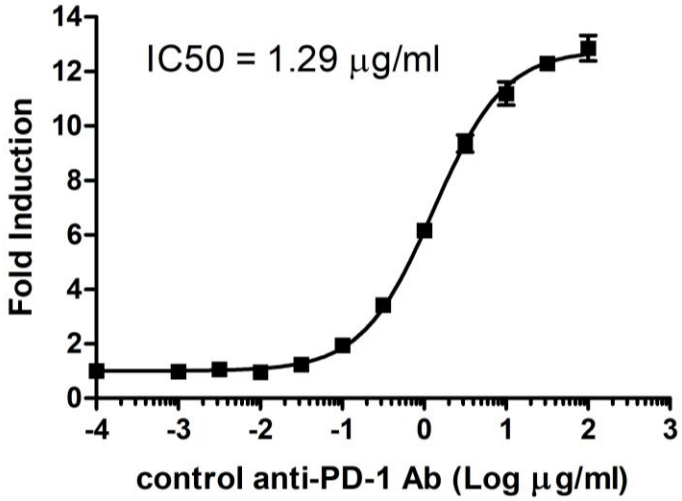
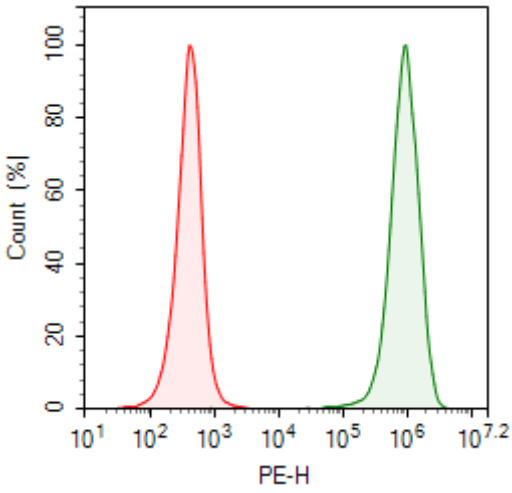


Figure 2. FACS analysis of cell surface expression of PD-L2 in PD-L2 / TCR activator PD-L2 / TCR activator CHO cells.
Flow cytometry using PE-conjugated anti-human PD-L2 antibody (Biolegend #329605) to detect PD-L2 surface expression on either PD-L2 / TCR activator PD-L2 / TCR activator CHO cells (green) or parental CHO-K1 cells (red).

FACS analysis: PD-L2 / TCR activator CHO with PE labeled anti-PD-L2



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Sequence
hPD-L2 sequence (accession number NM_025239)

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MIFLLLMLSLELQLHQIAALFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITASLQKVENDTSPHRERATLLE
EQLPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKYLTLKVKASYRKINTHILKVPETDEVELTCQATGYPLAEVS
WPNVSVPANTSHSRTPEGLYQVTSVLRLKPPPGRNFSCVFWNTHVRELTLASIDLQSQMEPRTHPTWLLHIFIPFCI
IAFIFIATVIALRKQLCQKLYSSKDTTKRPVTTTKREVNSAI
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License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

RELATED PRODUCTS:

Product	Cat. #	Size
PD-1/NFAT Reporter-Jurkat cell line	60535	2 vials
PD-L1 / TCR Activator - CHO Cell Line	60536	2 vials
NFAT Reporter – Jurkat cell line	60621	2 vials
TCR activator-CHO cell line	60539	2 vials
Anti-PD-1 neutralizing antibody	71120	100 µg
Anti-PD-L1 neutralizing antibody	71213	100 µg
TCR activator / PD-L1 expression kit	60610	500 rxns
TCR activator / PD-L2 expression kit	60620	500 rxns
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Human PD-1 (CD279), Fc fusion	71106	100 µg
Human PD-1, FLAG-Avi-His-tag	71198	50 µg
Human PD-L1 (CD274), Fc fusion	71104-1	50 µg
Human PD-L2 (CD273), Fc fusion	71107	100 µg
Human PD-1, Fc fusion, Biotin-labeled	71109	50 µg
Human PD-L1, Fc fusion, Biotin-labeled	71105	50 µg
Anti-PD-L1 Antibody, PE-labeled	71128	50 µg
Thaw Medium 3	60186	100 ml
Growth Medium 3A	60188	500 ml