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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

PD-L1 (Mouse) / TCR Activator - CHO Recombinant Cell Line
Cat. #: 79763

PRODUCT DESCRIPTION:
Recombinant CHO-K1 cells constitutively expressing mouse PD-L1 (Programmed Cell Death 1 Ligand 1, CD274**,** B7 homolog 1 (B7- H1), GenBank accession # NM_021893) 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. The PD-1 ligands are found on most cancers, and 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 mouse PD-1 signaling in a cellular context
- Screen mouse PD-L1 antibodies for binding affinity
- Characterize the biological activity of mouse PD-1 interactions with PD-L1

FORMAT:
Each vial contains 2.5 x 10^6 cells in 1 ml of 90% FBS, 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 Bioscience #60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

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Growth Medium 3A (BPS Bioscience #60188): Thaw Medium 3 (BPS Bioscience #60186) plus 1 mg/ml of Geneticin (Life Technologies #11811031) and 500 µg/ml of Hygromycin B (Hyclone #SV30070.01).

To ensure recombinant expression cells should be grown at 37°C with 5% CO_2 using Growth Medium 3A. Mouse PD-L1 / 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 and Hygromycin B)**, spin down cells, re-suspend cells in pre-warmed Thaw Medium 3 **(no Geneticin and Hygromycin B)**, transfer re-suspended cells to T25 flask and culture in 37°C CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 **(no Geneticin and Hygromycin B)**, and continue growing culture 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 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 culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 3A and transfer to a tube, spin down cells, re-suspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:10 to 1:20 twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from 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 cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 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 mouse PD-L1 in CHO-K1 cells was confirmed by FACS. The functionality of the cell line was validated using a mouse PD-1:PD-L1 cell-based assay. In this assay, Jurkat T cells expressing NFAT reporter with constitutive expression of mouse PD-1 (mouse PD-1/NFAT Reporter/Jurkat, BPS Bioscience #79762) are used as effector cells; TCR activator / mouse PD-L1- CHO cells are used as target cells. When these two cells are co-cultivated, TCR complexes on effector cells are activated by TCR activator on target cells, resulting in expression of the NFAT luciferase reporter. However, PD-1 and PD-L1 ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by anti-mouse PD-1 or anti-mouse PD-L1 antibodies. Mouse PD-1/PD-L1 neutralizing antibodies block PD-1:PD-L1 interaction and promote T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter.

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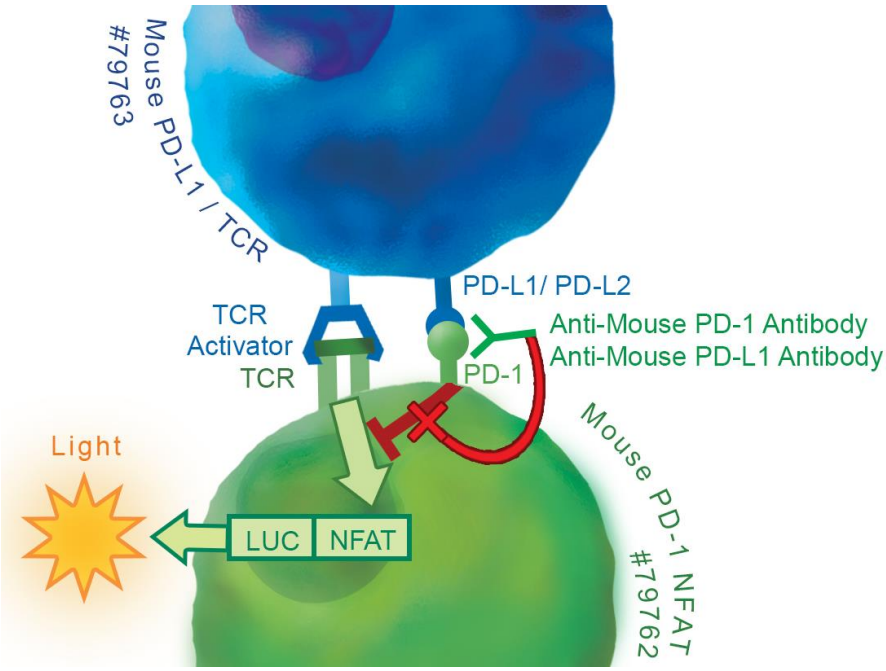
ASSAY PRINCIPLE



MATERIALS REQUIRED BUT NOT SUPPLIED:

- Mouse PD-1/NFAT reporter-Jurkat cell line (BPS Bioscience #79762)
- Assay medium: Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 3A (BPS Bioscience #60188)
- Anti-mouse PD-1 neutralizing antibody: Bioxcell #BP0273, clone#29F.1A12
- Anti-mouse PD-L1 neutralizing antibody (BPS Bioscience #71213)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer
- Thaw Medium 3 (BPS Cat. #60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

PROTOCOL:

1. Harvest Mouse PD-L1 / TCR activator -CHO cells from culture and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 100 µl of Thaw Medium 3, BPS Bioscience #60186 (growth medium without Geneticin and

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Hygromycin B). Incubate cells at 37°C in a CO_2 incubator for overnight. Cells should reach ~80% confluency on the next day (cells should not reach confluency in this step).

2. Next day, prepare serial dilution of anti-mouse PD-1 antibody or anti-mouse PD-L1 antibody in Thaw Medium 2, BPS Bioscience #60184 (assay medium) (the concentration of antibody here is 2x of the final treatment concentration of antibody). Harvest the mouse PD-1/NFAT-reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute cells to 4 x 10^5 / ml in assay medium.

   To test anti-PD-1 antibody, preincubate the PD-1/NFAT Reporter- Jurkat cells (4 x 10^5 / ml) with diluted anti-PD-1 antibody (1:1 in volume) for 30 min. After incubation, remove the medium from *PD-L1/*TCR activator-CHO cells and add 100 µl of PD-1/NFAT reporter – Jurkat cells / anti-PD-1 antibody mixture to the wells. (Note: *Thoroughly mix the PD-1/NFAT Reporter- Jurkat cells with antibody before adding to PD-L1/TCR activator-CHO cells.*)

   To test the anti-PD-L1 antibody, remove the medium from *PD-L1/*TCR activator-CHO cells, add 50 µl of diluted anti-PD-L1 antibody to the wells and incubate for 30 min. After incubation, add 50 µl of PD-1/NFAT Reporter- Jurkat cells (4 x 10^5 / ml) to the wells. (Note: *Thoroughly mix the PD-1/NFAT Reporter- Jurkat cells before adding to PD-L1/TCR activator-CHO cells.)*

   Final cell density of mouse PD-1/NFAT Reporter- Jurkat cells is 2 x 10^4 /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° in a CO_2 incubator for 5 to 6 hours.

3. After ~5 to 6-hour incubation, prepare luciferase reagents using the ONE-Step luciferase assay system, according to recommended instructions. 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.

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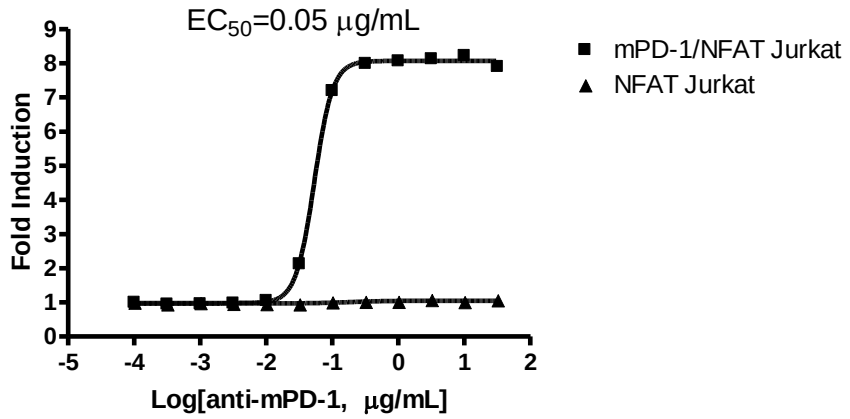

Figure 1. Cell Characterization Using a Mouse PD-1 Neutralizing Antibody. Mouse PD-L1/ TCR activator-CHO cells were seeded in 96-well plate. The next day, mouse /PD-L1/TCR activator -CHO cells were incubated with anti-mouse PD-1 neutralizing antibody (Bioxcell #BP0273, clone#29F.1A12) and mouse PD-1/NFAT Reporter-Jurkat cells (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.

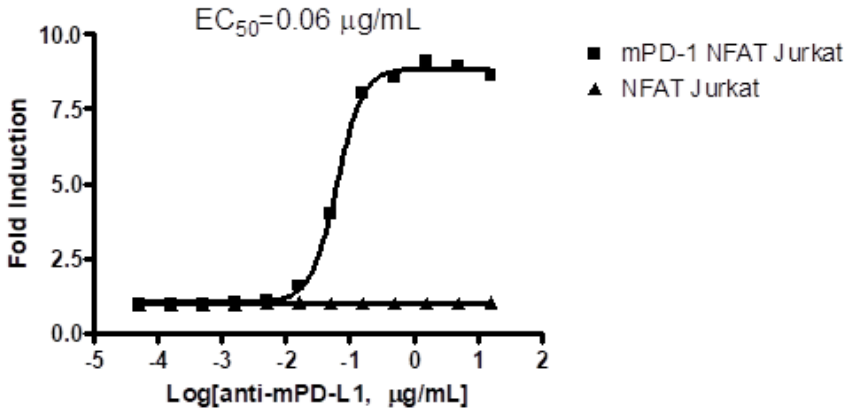

Figure 2. Cell Characterization Using a Mouse PD-L1 Neutralizing Antibody. Mouse PD-L1/TCR activator -CHO cells were seeded in 96-well plate. The next day, Mouse PD-L1/ TCR activator-CHO cells were incubated with anti-PD-L1 neutralizing antibody and mouse PD-1/NFAT Reporter-Jurkat cells (or control NFAT Reporter – Jurkat cells, BPS Bioscience #60621). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to 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.

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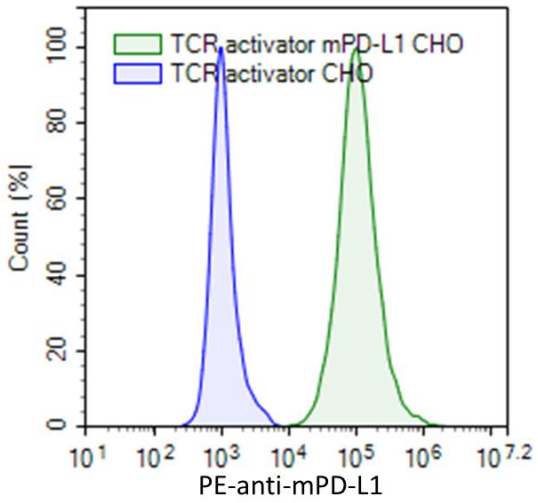

Figure 3. FACS analysis of cell surface expression of mouse PD-L1 in Mouse PD-L1/TCR activator CHO cells. Mouse PD-L1/TCR activator-CHO cells or control TCR activator CHO cells were stained with PE-labeled anti-mouse PD-L1 antibody and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.

SEQUENCE:

mPD-L1 sequence (Genbank accession #NM_021893)

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQV
IQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITL
KVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNV
TSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNRTHWVLLGSILLFLIVVSTV
LLFLRKQVRMLDVEKCGVEDTSSKNRNDTQFEET

RELATED PRODUCTS:

Product	Cat. #	Size
Mouse PD-1/NFAT Reporter-Jurkat cell line	79762	2 vials
PD-1/NFAT Reporter-Jurkat cell line	60535	2 vials
NFAT Reporter – Jurkat cell line	60621	2 vials
TCR activator-CHO cell line	60539	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Mouse PD-1[Biotinylated]:PD-L1 Inhibitor Assay Kit	72027	96 rxns

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