Description

PD-L1 (Mouse)/TCR Activator CHO Cell Line is a CHO-K1 cell line 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. Surface expression of PD-L1 was confirmed by flow cytometry, and the cell line was functionally validated in a mouse PD-1:PD-L1 cell-based assay.

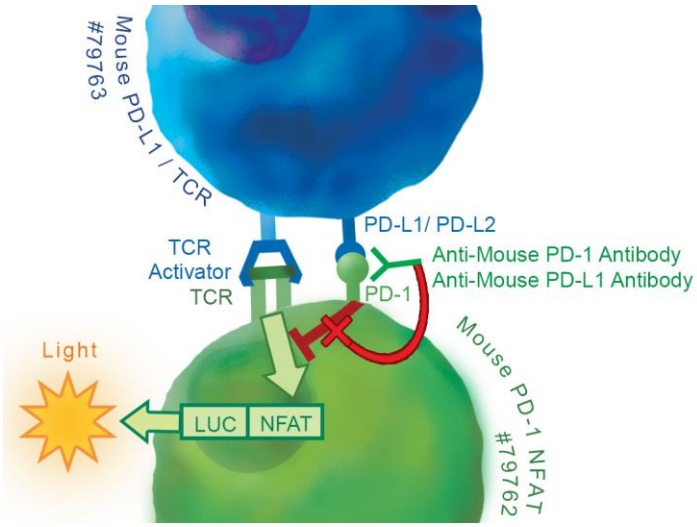

Figure 1: Mechanism of action of PD-L1 (Mouse)/TCR Activator CHO Cell Line in combination with the Mouse PD-1/NFAT reporter-Jurkat cell line. Jurkat T cells expressing NFAT reporter with constitutive expression of mouse PD-1 (mouse PD-1/NFAT Reporter Jurkat Cell Line, BPS Bioscience #79762) act as effector cells. When co-cultivated, TCR complexes on mouse PD-1/NFAT Reporter Jurkat cells are activated by the TCR activator of PD-L1 (Mouse)/TCR Activator CHO Cell Line, 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.

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 in 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.

Application

- Screen and validate activators or inhibitors of mouse PD-1 signaling in a cellular model.
- Characterize the biological activity of mouse PD-1 interactions with PD-L1.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line
CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, adherent

Mycoplasma Testing
The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 3	BPS Bioscience #60186
Growth Medium 3A	BPS Bioscience #60188

Materials Required for Cellular Assays

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Mouse PD-1/NFAT Reporter-Jurkat Cell Line	BPS Bioscience #79762
NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60621
Anti-mouse PD-1 Neutralizing Antibody	Bioxcell #BP0273
Anti-mouse PD-L1 Neutralizing Antibody	Fisher Scientific #50-146-65
96-well tissue culture-treated white clear-bottom assay plate	
One-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations
For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

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

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

Assay Medium:
Thaw Medium 2: RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Cell Culture Protocol

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.
Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 3 to the conical tube containing the cells. Thaw Medium 3 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 3.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to be split.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3A.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3A and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 to 1:20 twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 3 and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

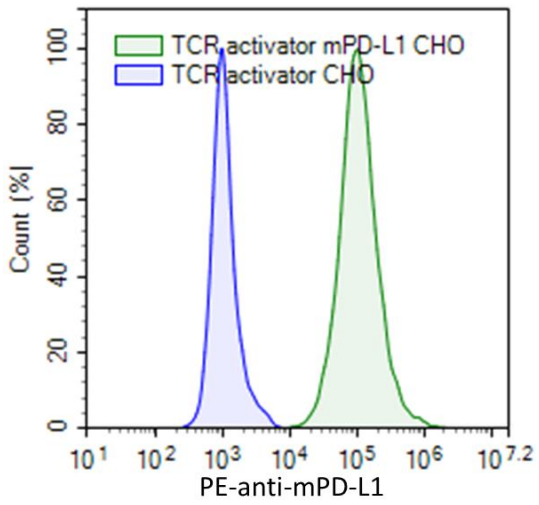

Figure 2: Cell surface expression of mouse PD-L1 in Mouse PD-L1 (Mouse)/TCR Activator CHO Cell Line.
Mouse PD-L1 (Mouse)/TCR Activator CHO cells (Green) and control TCR Activator CHO-K1 cells (Blue) were stained with PE-labeled anti-mouse PD-L1 antibody and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

Functional Validation

- The following assays are designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.
- The assay should be performed in triplicate.
- The assay should include cell-free wells and NFAT Reporter (Luc)– Jurkat Recombinant Cells as controls.

Assay Medium: Thaw Medium 2.

A. Dose response of Mouse PD-L1 (Mouse)/TCR Activator CHO Cell Line to anti-PD-1 antibody

1. Seed Mouse PD-L1 (Mouse)/TCR Activator CHO cells into a white clear-bottom 96-well microplate at a density of ~35,000 cells per well in 100 µl of Thaw Medium 3. Leave a couple of wells empty for use as the cell-free control.

2. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

   Note: Cells should be at approximately 80% confluency after 16-24 hours. They should not be fully confluent.

3. The next day, prepare a serial dilution of anti-mouse PD-1 antibody in Assay Medium at 2-fold the final concentrations to be tested.

4. Dilute PD-1/NFAT Reporter Jurkat and NFAT Luciferase Reporter Jurkat cells to 0.4 x 10^6 cells/ml in Assay Medium.

5. Incubate the diluted PD-1/NFAT Reporter Jurkat cells and NFAT Luciferase Reporter Jurkat cells with the diluted anti-mouse PD-1 antibody solutions (1:1) for 30 minutes.

6. Remove the spent media from the wells with Mouse PD-L1 (Mouse)/TCR Activator CHO cells.

7. Add 100 µl of diluted Jurkat cells/ anti-PD1 mixture to each well with Mouse PD-L1 (Mouse)/TCR Activator CHO cells.

8. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).

9. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

10. Add 100 µl of the ONE-Step™ Luciferase reagent per well.

11. Rock gently at Room Temperature (RT) for ~15 minutes.

12. Measure luminescence using a luminometer.

13. 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.

$$Fold\ induction = \frac{average\ Lum\ sample - average\ backgound}{average\ Lum\ control - average\ background}$$

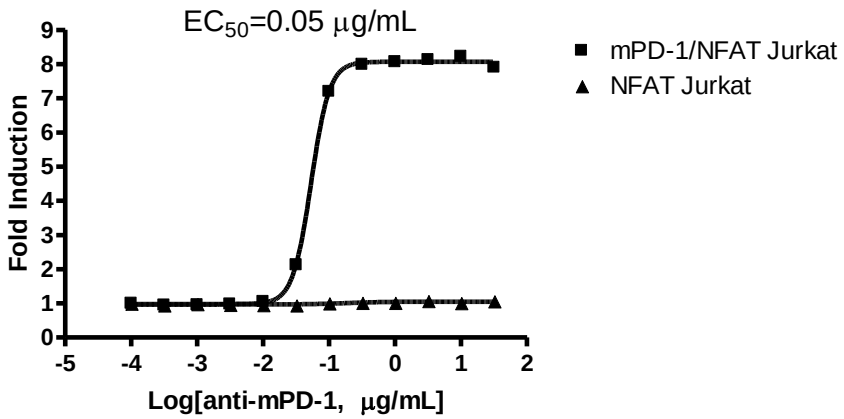

Figure 3: Dose response profile of cell activation by PD-1 Neutralizing Antibody in Mouse PD-L1 (Mouse)/TCR Activator CHO Cell Line.
Mouse PD-L1/ TCR Activator CHO cells were seeded in 96-well plate. The next day cells were incubated with anti-mouse PD-1 neutralizing antibody and mouse PD-1/NFAT Reporter Jurkat cells or control NFAT Luciferase Reporter Jurkat cells. After incubation, NFAT activity was measured with ONE-Step™ Luciferase Assay System. 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.

B. Dose response of Mouse PD-L1 (Mouse)/TCR Activator CHO Cell Line to anti-PD-L1 antibody

1. Seed Mouse PD-L1 (Mouse)/TCR Activator CHO cells into a white clear-bottom 96-well microplate at a density of ~35,000 cells per well in 100 µl of Thaw Medium 3. Leave a couple of wells empty for use as the cell-free control.

2. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

   Note: Cells should be at approximately 80% confluency after 16-24 hours. They should not be fully confluent.

3. The next day, prepare a serial dilution of anti-mouse PD-L1 antibody in Assay Medium at 2-fold the final concentrations to be tested.

4. Dilute PD-1/NFAT Reporter- Jurkat and NFAT Luciferase Reporter Jurkat cells to 0.4 x 10^6 cells/ml in Assay Medium.

5. Remove the spent media from the wells with Mouse PD-L1 (Mouse)/TCR Activator CHO cells.

6. Add 50 µl of diluted anti-PD-L1 antibodies to each well with Mouse PD-L1 (Mouse)/TCR Activator CHO cells and incubate for 30 minutes.

7. Add 50 µl of the diluted PD-1/NFAT Reporter Jurkat cells or NFAT Luciferase Reporter Jurkat Recombinant cells.

8. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).

9. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

10. Add 100 µl of the ONE-Step™ Luciferase reagent per well.

11. Rock gently at Room Temperature (RT) for ~15 minutes.

12. Measure luminescence using a luminometer.

13. 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.

$$Fold\ induction = \frac{average\ Lum\ sample - average\ backgound}{average\ Lum\ control - average\ background}$$

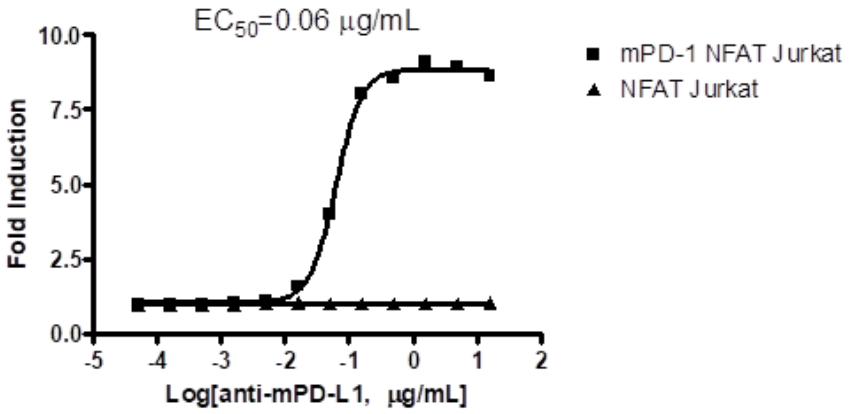

Figure 4: Dose response profile of cell activation by PD-L1 Neutralizing Antibody in Mouse PD-L1 (Mouse)/TCR Activator CHO Cell Line.
Mouse PD-L1/ TCR Activator CHO cells were seeded in 96-well plate. The next day cells were incubated with anti-mouse PD-L1 neutralizing antibody and mouse PD-1/NFAT Reporter Jurkat cells or control NFAT Reporter Jurkat cells. After incubation, NFAT activity was measured with ONE-Step™ Luciferase Assay System. 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.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*.*

Sequence
> mPD-L1 sequence (Genbank accession #NM_021893)
MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQVIQFVAGEEDLKPQHSNFRGRASL
PKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPV
SGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNRTHWVLLGSILLFLIVVSTVLLFLR
KQVRMLDVEKCGVEDTSSKNRNDTQFEET

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
TCR Activator CHO Cell Line	60539	2 vials
PD-1 CHO Cell Line	78530	2 vials
PD1:PD-L1 TR-FRET Assay	72032	96 reactions
PD1:PD-L1 Cell Based Inhibitor Screening Assay Kit	79377	96 reactions
Anti-PD-L1 Antibody, PE-Labeled	71128	50 µg/100 µg