Description

Recombinant CHO-K1 cells constitutively expressing human PD-L1 (Programmed Cell Death 1 Ligand 1, CD274, B7 homolog 1 (B7-H1), GenBank accession #NM_014143) 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 the surface of most cancer cells, and their interaction with receptor PD-1 inhibits T cell activity and allows cancer cells to escape immune surveillance. This pathway is also involved in regulating autoimmune responses. Therefore, these proteins (termed immune checkpoints) are promising therapeutic targets for many types of cancer, as well as multiple sclerosis, arthritis, lupus, and type I diabetes. Checkpoint inhibitors have remarkable efficacy in a wide range of cancer types and have revolutionized cancer treatment. PD-1 inhibitors nivolumab, pembrolizumab, cemiplimab and PD-L1 inhibitors atezolizumab, avelumab, and durvalumab are all FDA-approved drugs for immunotherapy.

Application

- Screen for inhibitors of PD-1 or PD-L1 signaling in a cellular context
- Characterize the biological activity of PD-1 interactions with PD-L1

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of 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 Assay

Name	Ordering Information
PD-1/NFAT reporter Jurkat cell line	BPS Bioscience #60535
Thaw Medium 2	BPS Bioscience #60184
Anti-PD-1 neutralizing antibody	BPS Bioscience #71120
Anti-PD-L1 neutralizing antibody	BPS Bioscience #71213
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white, clear-bottom assay plate	
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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 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

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience, #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

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

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 3 **(no Geneticin and Hygromycin B).**

   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. 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 **(no Geneticin and Hygromycin B).**
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 **(no Geneticin and Hygromycin B)**, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3A **(contains Geneticin and Hygromycin B)**

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3A and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium **3A (contains Geneticin and Hygromycin B)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 to 1:20 twice a week.

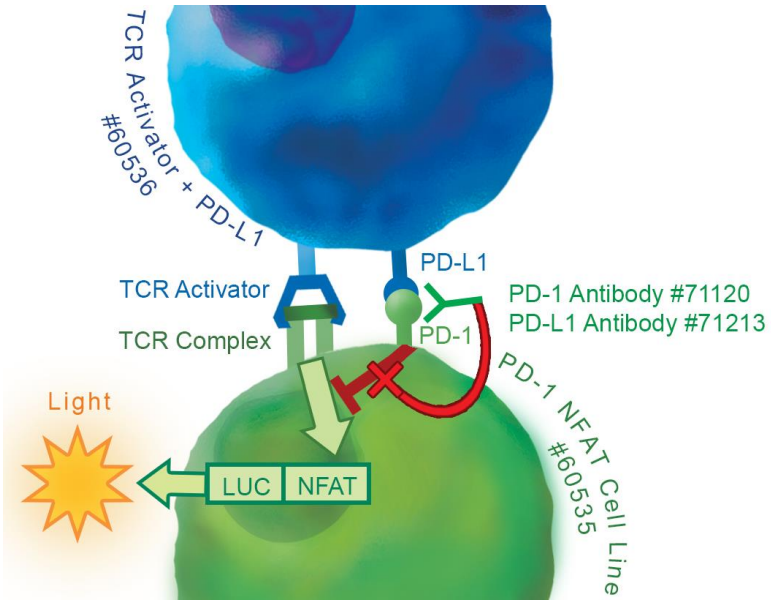
Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3A 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.

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

Assay Principle

This co-culture assay is designed to observe the consequences of PD-L1/PD-1 interaction on Jurkat T cell activation. The TCR activator present at the surface of TCRa/PD-L1 CHO cells stimulates TCR in Jurkat T cells, whereas overexpression of PD-L1 on the CHO cell line engages Jurkat PD-1, which blocks TCR activation signals and prevents activation of NFAT. Addition of a neutralizing anti-PD-1 or anti-PD-L1 antibody to the co-culture releases the PD-L1/PD-1 brake and results in TCR activation and increased NFAT activity, which translates into increased luciferase reporter signal.



Cellular Assay Protocol

1. Seed TCR activator/PD-L1 CHO 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. Incubate the cells at 37°C in a CO2 incubator overnight. Cells should reach ~80% confluency on the next day (cells should not be confluent in this step).

2. The next day, prepare a serial dilution of anti-PD-1 antibody or anti-PD-L1 antibody in Thaw Medium 2 (50 ml/well needed). The concentration of antibody in the serial dilution should be 2-fold higher than the desired final concentration. Each treatment should be performed at least in triplicate.

3. Harvest the PD-1/NFAT Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of $4x10^5$/ml (50 ml/well). The final desired number of PD-1/NFAT Reporter Jurkat cells is 20,000 cells/well.

    a. **To test an anti-PD-1 antibody**, preincubate the PD-1/NFAT Reporter Jurkat cells ($4x10^5$/ml) with the diluted anti-PD-1 antibody (50 ml of cells + 50 ml of antibody) for 30 minutes at 37°C.

    Remove the medium from the TCR activator/PD-L1 CHO cells and add 100 µl of the mix PD-1/NFAT Reporter Jurkat cells + anti-PD-1 antibody to the wells containing the CHO cells.
    Note: Ensure that the PD-1/NFAT Reporter Jurkat cells + antibody solution is well mixed before adding to the TCR activator/PD-L1 CHO cells.

b. **To test an anti-PD-L1 antibody**, remove the medium from the TCR activator/PD-L1 CHO cells, add 50 µl of the diluted anti-PD-L1 antibody to the wells and incubate for 30 minutes at 37°C. After incubation, add 50 µl of PD-1/NFAT Reporter Jurkat cells ($4x10^5$/ml) to the wells.

4. To determine background luminescence, add 100 µl of Thaw Medium 2 to cell-free control wells.

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

6. After incubating for 5 to 6 hours, perform a 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 ~15 minutes. Measure luminescence using a luminometer.

7. 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 is the average background-subtracted luminescence of antibody-treated wells divided by the average background-subtracted luminescence of untreated control wells.

$$Fold\ induction = \frac{luminescence\ of\ treated\ wells - background}{luminescence\ of\ untreated\ well - background}$$

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry. To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay.

Experimental Results

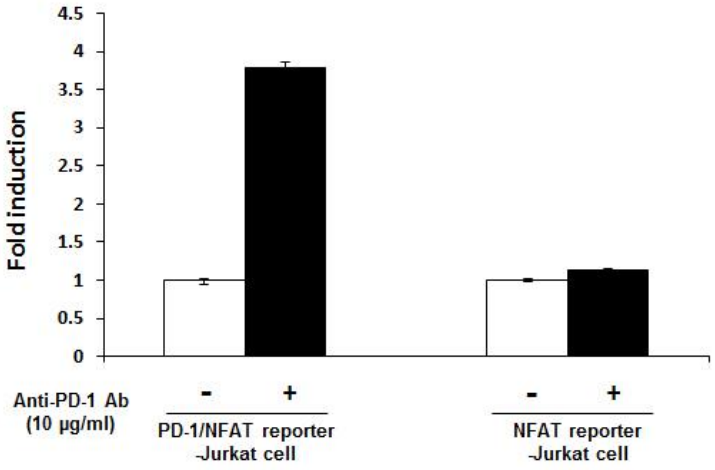


Figure 1. Effect of an anti-PD-1 Neutralizing Antibody on NFAT activation in PD-1/NFAT Reporter Jurkat cells. A co-culture assay was performed with the TCR Activator PD-L1 CHO cell line (BPS Bioscience #60536) as described in the protocol above. Addition of the anti-PD-1 neutralizing antibody (BPS Bioscience #71120) increased NFAT-induced luciferase reporter activity in PD-1/NFAT Reporter Jurkat cells that overexpress PD-1 (BPS Bioscience #60535), but not in NFAT Reporter Jurkat cells, co-cultured with TCR activator/PD-L1 CHO cells.

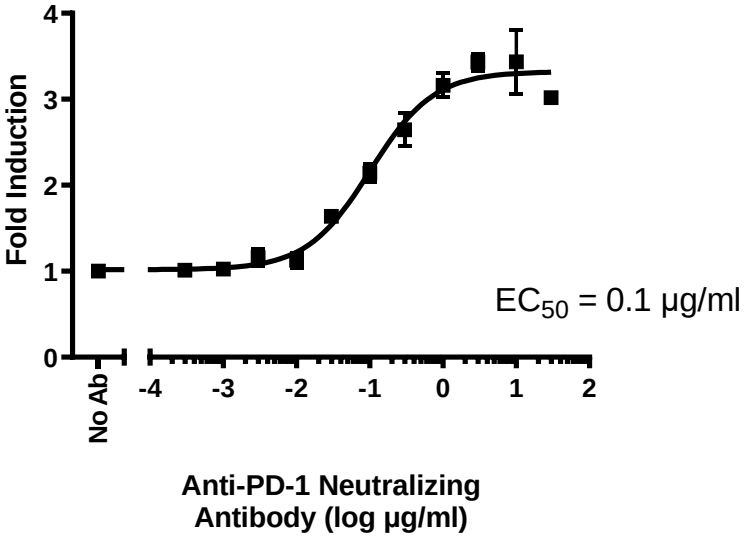


Figure 2. Dose-response of an anti-PD-1 Neutralizing Antibody on NFAT activation in the PD-1/NFAT Reporter Jurkat cells. A co-culture assay was performed with the TCR Activator/PD-L1 CHO cell line (BPS Bioscience #60536) as described in the protocol above, in the presence of increasing concentrations of anti-PD-1 neutralizing antibody (BPS Bioscience #71120). Addition of the anti-PD-1 neutralizing antibody to PD-1/NFAT Reporter-Jurkat cells (BPS Bioscience #60535) co-cultured with TCR activator/PD-L1-CHO cells resulted in the dose-dependent activation of NFAT in the Jurkat cells.

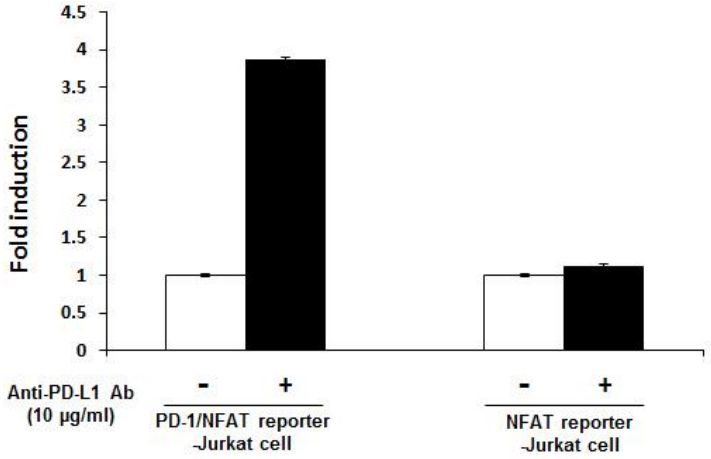


Figure 3. Effect of an anti-PD-L1 Neutralizing Antibody on NFAT activation in the PD-1/NFAT reporter Jurkat cells. A co-culture assay was performed with the TCR-Activator/PD-L1 CHO cell line (BPS Bioscience #60536) in the presence of an anti-PD-L1 neutralizing antibody (BPS Bioscience #71213). Addition of the antibody resulted in the activation of NFAT in PD-1/NFAT Reporter Jurkat cells that overexpress PD-1 (BPS Bioscience #60535), but not in NFAT Reporter Jurkat cells, co-cultured with TCR activator/PD-L1-CHO cells.

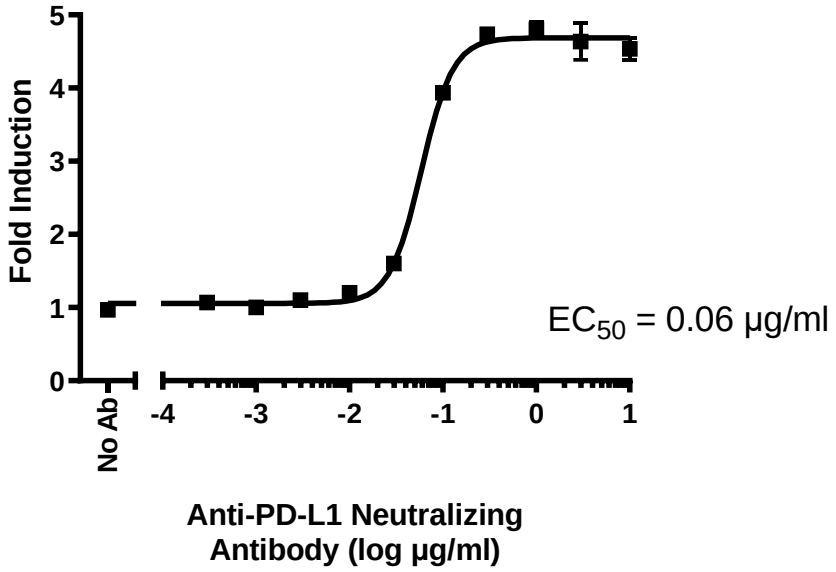


Figure 4. Effect of an PD-L1 Neutralizing Antibody on NFAT activation in the PD-1/NFAT reporter Jurkat cells. A co-culture assay was performed with the TCR-Activator PD-L1 CHO cell line (BPS Bioscience #60536) as described in the protocol above, in the presence of increasing concentrations of anti-PD-L1 neutralizing antibody (BPS Bioscience #71213). Addition of the antibody resulted in the activation of NFAT and dose-dependent increase in luciferase activity in the PD-1/NFAT Reporter Jurkat cells (BPS Bioscience #60535) co-cultured with TCR activator/PD-L1-CHO cells.

Sequence
Human PD-L1 sequence (accession number NM_014143)

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRA
RLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLR
KGRMMDVKKCGIQDTNSKKQSDTHLEET

References
Sasca D, *et al.* (2019) *Blood* **133**: 2305-2319.

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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*
PD-1/NFAT Reporter Jurkat Recombinant Cell Line	60535	2 vials
NFAT Reporter (Luc) Jurkat Recombinant Cell Line	60621	2 vials
TCR Activator CHO Recombinant Cell line	60539	2 vials
Anti-PD-1 Neutralizing Antibody	71120	100 µg
Anti-PD-L1 (CD274) Neutralizing Antibody	71213	100 µg
PD-L1/TCR Activator Mammalian Expression Kit	60610	500 reactions
PD-L2/TCR Activator Mammalian Expression Kit	60620	500 reactions
ONE-Step™ Luciferase Assay System	60690	10 mg/100 mg
PD-1 (CD279), Fc fusion (Human)	71106	100 µg
PD-1, FLAG-Avi-His-tag (Human) HiP™	71198	50 µg
PD-L1 (CD274), Fc fusion (Human) HiP™	71104	50 µg
PD-L1 (CD274), FLAG-tag (Human) HiP™	71183	50 µg
PD-L2 (CD273), Fc fusion (Human) HiP™	71107	100 µg
PD-1 (CD279), Fc fusion, Biotin-labeled (Human) HiP™	71109	25 µg
PD-L1 (CD274), Fc fusion, Biotin-labeled (Human) HiP™	71105	25 µg
Anti-PD-L1 Antibody, PE-labeled	71128	50 µg