Description

PD-L1/TCR Activator-CHO Recombinant Cell Line is a CHO-K1 cell line constitutively expressing human PD-L1 (Programmed Cell Death 1 Ligand 1, CD274 or B7 homolog 1 (B7-H1), GenBank accession #NM_014143) and an engineered TCR (T cell receptor) activator.

This cell line was functionally validated with Anti-PD-1 and Anti-PD-L1 neutralizing antibodies in co-culture assays.

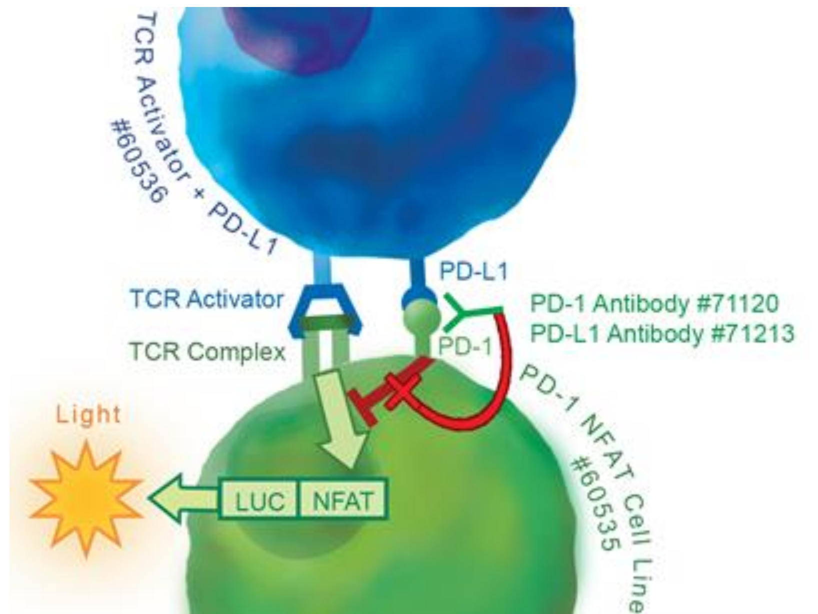

Figure 1: Mechanism of action of PD-L1/TCR Activator CHO Recombinant Cell Line in a co-culture assay.
The TCR activator present at the surface of PD-L1/TCR Activator CHO cells stimulate TCR in Jurkat T cells, whereas overexpression of PD-L1 on the CHO cell line engages Jurkat PD-1, blocking TCR activation signaling and preventing 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 complex and results in TCR activation and increased NFAT activity, which translates into increased luciferase reporter signal.

Background

PD-L1 and PD-L2 binding to PD-1, a receptor expressed on T-cells, negatively regulates immune responses. The PD-1 ligands PD-L1 and PD-L2 are found on the surface of most cancer cells, and their interaction with the 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. The PD-1 inhibitors nivolumab, pembrolizumab, cemiplimab and PD-L1 inhibitors atezolizumab, avelumab, and durvalumab are all FDA-approved drugs for immuno-therapy.

Application

- Screen for activators or inhibitors of PD-1 signaling in a cellular model.
- Characterize the biological activity of PD-1 and interaction with its ligands.

BPS Bioscience
Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 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
Thaw Medium 2	BPS Bioscience #60184
PD-1/NFAT Reporter Jurkat Recombinant Cell Line	BPS Bioscience #60535
NFAT Reporter (Luc)-Jurkat Recombinant Cell Line	BPS Bioscience #60621
Anti-PD-1 Neutralizing Antibody	BPS Bioscience #71120
Nivolumab (anti-PD-1)	SelleckChem #A2002
Anti-PD-L1 (CD274) Neutralizing Antibody	BPS Bioscience #71213
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℃ 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.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60535):
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 0.25% 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 0.25% 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 Cell Freezing Medium (BPS Bioscience #79796) at ~2×10^6 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

- This co-culture assay is designed to analyze the effect of PD-L1/PD-1 interaction on Jurkat T cell activation.
- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assays should include “Cell-Free Control”, "Untreated Control” and “Treated” conditions.
- The use of NFAT Luciferase Reporter Jurkat Cell Line as control is recommended.

A. Testing of Anti-PD-1 Antibody on a co-culture of PD-1/NFAT Reporter Jurkat Recombinant Cell Line and PD-L1 /TCR Activator-CHO Recombinant Cell Line.

1. Seed PD-L1/TCR Activator CHO cells at a density of 35,000 cells per well in 100 µl of Thaw Medium 3 into a white, clear-bottom 96-well microplate. Leave a few wells empty to use as the “Cell-Free Control” (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator overnight.

   Note: Cells should reach ~80% confluency on the next day (cells should not reach full confluence in this step).

3. Prepare a serial dilution of anti-PD-1 antibody in Thaw Medium 2 at 2x the final treatment concentration (50 µl/well needed).

4. Harvest PD-1/NFAT Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of 4 x 10^5/ml (50 µl/well).

5. Preincubate the PD-1/NFAT Reporter Jurkat cells (4 x 10^5/ml) with the diluted anti-PD-1 antibody (1:1 in volume) for 30 minutes at 37°C (100 µl mix/well).

6. Remove the medium from PD-L1-/TCR Activator CHO cells and add 100 µl of the PD-1/NFAT Reporter Jurkat cells/anti-PD-1 antibody mixture to the “Treated” wells.

   Note: Mix the PD-1/NFAT Luciferase Reporter Jurkat cells with antibody thoroughly before adding to the CHO cells.

7. Add 50 µl of PD-1/NFAT Reporter Jurkat cells (4 x 10^5/ml) (no antibody) and 50 µl of Thaw Medium 2 to the “Untreated Control” wells.

8. Add 100 µl of Thaw Medium 2 to the “Cell-Free Control” wells (for determining background luminescence).

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

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

11. Rock gently at room temperature 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.

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

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

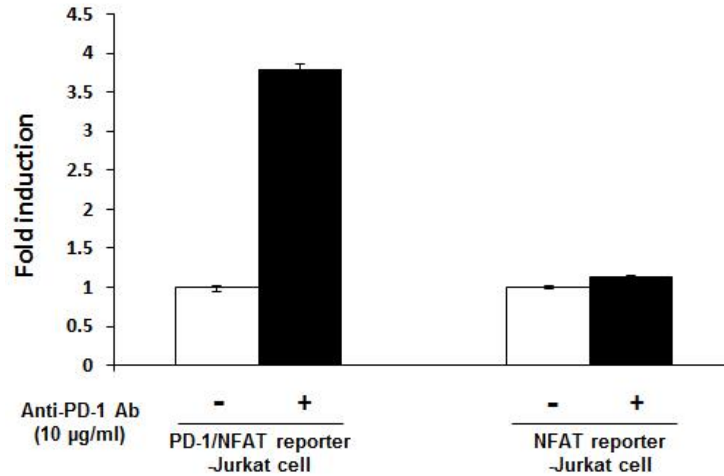

Figure 2. Effect of Anti-PD-1 Neutralizing Antibody on NFAT activation in the PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured with PD-L1/TCR Activator-CHO Recombinant Cell Line.
A co-culture assay was performed with PD-L1/TCR Activator CHO cells as described in the protocol above. Addition of Anti-PD-1 Neutralizing Antibody increased NFAT-induced luciferase reporter activity in PD-1/NFAT Reporter Jurkat cells, but not in NFAT Reporter Jurkat cells, when co-cultured with PD-L1/TCR Activator CHO cells.

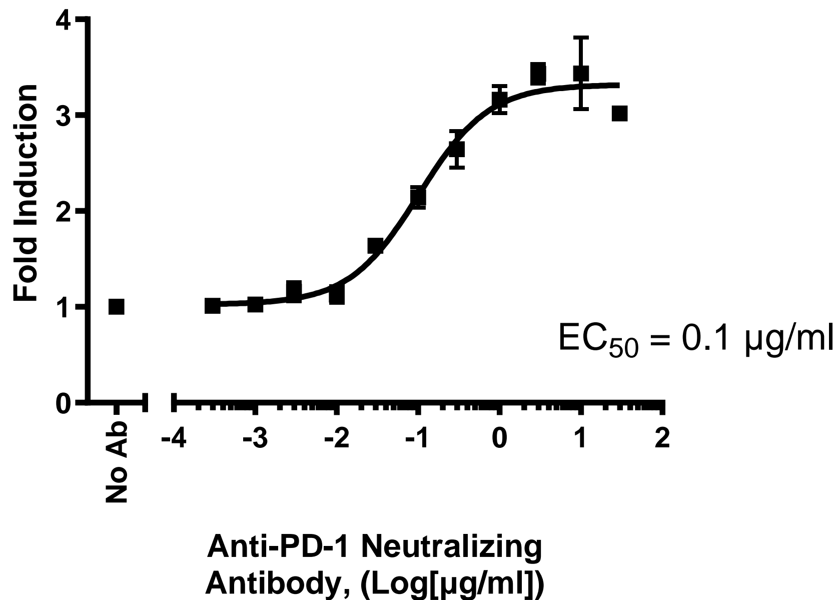

Figure 3. Dose-response curve of PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured with PD-L1/TCR Activator-CHO Recombinant Cell Line to Anti-PD-1 Neutralizing Antibody.
A co-culture assay was performed with PD-L1/TCR Activator-CHO cells as described in the protocol above, in the presence of increasing concentrations of Anti-PD-1 Neutralizing Antibody. Addition of Anti-PD-1 Neutralizing Antibody to PD-1/NFAT Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells resulted in the dose-dependent activation of NFAT.

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

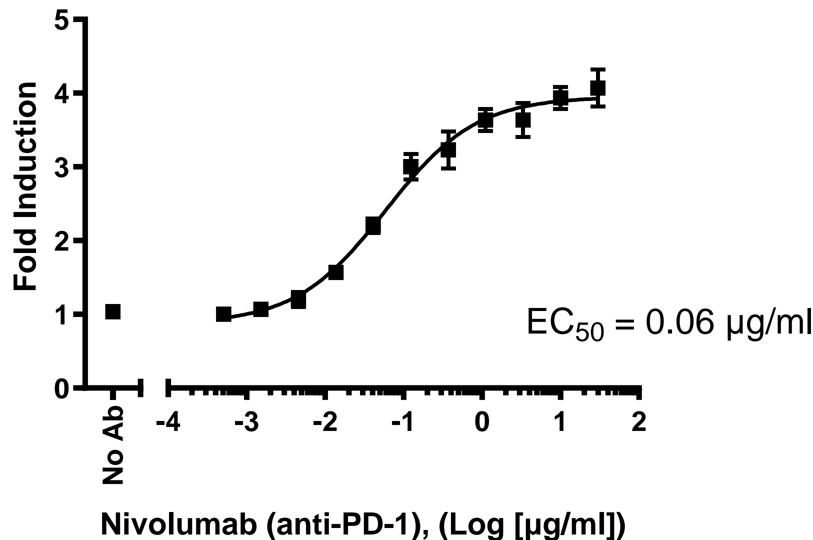

Figure 4. Dose-response curve of PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured with PD-L1/TCR Activator-CHO Recombinant Cell Line to Nivolumab (anti-PD-1).
A co-culture assay was performed with the PD-L1/TCR Activator - CHO cells as described in the protocol above, in the presence of increasing concentrations of Nivolumab. Addition of Nivolumab to PD-1/NFAT Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells resulted in the dose-dependent activation of NFAT.

B. Testing of Anti-PD-L1 Neutralizing Antibody on a co-culture of PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured and PD-L1/TCR Activator-CHO Recombinant Cell Line.

1. Seed PD-L1/TCR Activator CHO cells at a density of 35,000 cells per well in 100 µl of Thaw Medium 3 into a white, clear-bottom 96-well microplate. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator overnight.

   Note: Cells should reach ~80% confluency on the next day (cells should not reach full confluence in this step).

3. Prepare a serial dilution of anti-PD-L1 antibody in Thaw Medium 2 at the final treatment concentration (50 µl/well needed).

4. Harvest PD-1/NFAT Luciferase Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of 4 x 10^5/ml (50 µl/well).

5. Remove the medium from PD-L1-/TCR Activator CHO cells and add 50 µl of the anti-PD-L1 antibody dilution to the "Treated" wells.

6. Add 50 µl of Thaw Medium 2 to the "Untreated Control" wells.

7. Incubate for 30 minutes at at 37°C in a 5% CO_2 incubator.

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

8. Add 50 µl of PD-1/NFAT Luciferase Reporter Jurkat cells (4 x 10^5/ml) to the "Treated" and "Untreated Control" wells.

9. Add 100 µl of Thaw Medium 2 to the "Cell-Free Control" wells (for determining background luminescence).

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

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

12. Rock gently at room temperature for ~15 minutes.

13. Measure luminescence using a luminometer.

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

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

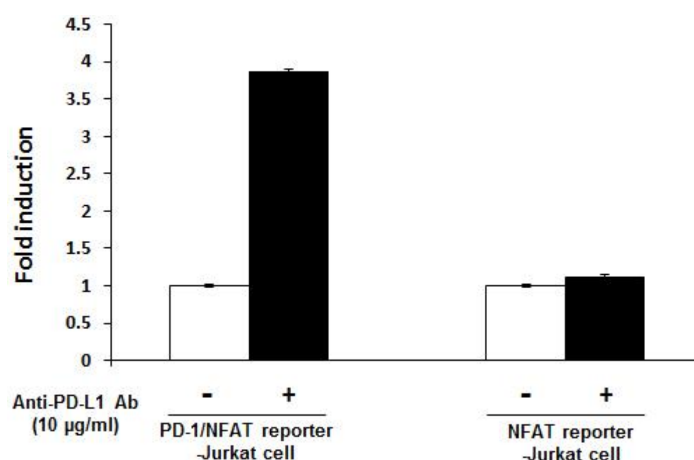

Figure 5. Effect of Anti-PD-L1 Neutralizing Antibody on NFAT activation in the PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured with PD-L1/TCR Activator-CHO Recombinant Cell Line.
A co-culture assay was performed with the PD-L1/TCR-Activator CHO *cells* in the presence of Anti-PD-L1 Neutralizing Antibody. Addition of the antibody resulted in the activation of NFAT in PD-1/NFAT Reporter Jurkat cells that overexpress PD-1, but not in NFAT Reporter Jurkat cells, when co-cultured with PD-L1/TCR Activator CHO cells.

BPS Bioscience
For research use only. Not for human, drug, diagnostic, or therapeutic use.
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

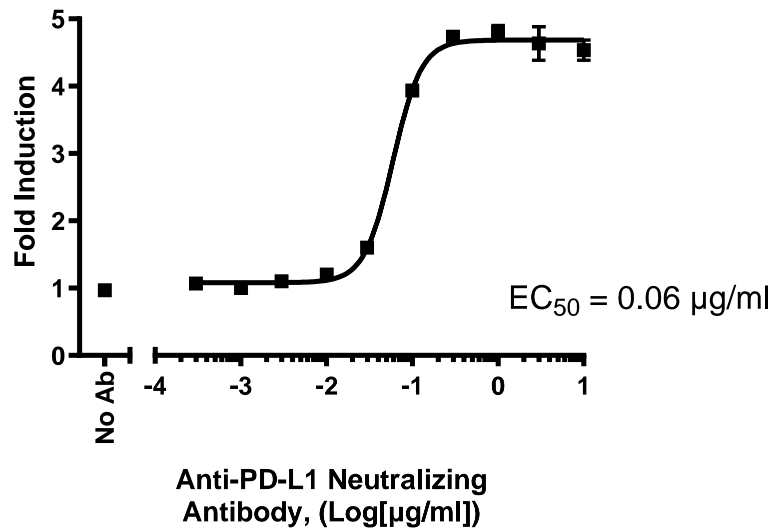

Figure 6. Dose response curve of PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured with PD-L1/TCR Activator-CHO Recombinant Cell Line to PD-L1 Neutralizing Antibody.
A co-culture assay was performed with the PD-L1/TCR-Activator CHO cells as described in the protocol above, in the presence of increasing concentrations of Anti-PD-L1 Neutralizing Antibody. Addition of the antibody resulted in the activation of NFAT and a dose-dependent increase in luciferase activity in the Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells.

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

Sequence
Human PD-L1 sequence (accession number NM_014143)

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRA
RLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLR
KGRMMDVKKCGIQDTNSKKQSDTHLEET

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

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

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 Recombinant Cell line	60539	2 vials
PD-1, FLAG-Avi-His-Tag (Human) HiP™	71198	50 µg
Anti-PD-L1 Antibody, PE-Labeled	71128	50 µg
PD1:PD-L1 TR-FRET Assay	72032	96 reactions
PD1:PD-L1 Cell Based Inhibitor Screening Assay Kit	79377	96 reactions

Version 030424

BPS Bioscience
For research use only. Not for human, drug, diagnostic, or therapeutic use.
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com