Description

PD-1/NFAT Reporter Jurkat Recombinant Cell Line is a Jurkat cell line that expresses human PD-1 (Programmed Cell Death 1, also known as PDCD1, SLEB2, CD279, GenBank Accession #NM_005018), and the firefly luciferase reporter under the control of NFAT response elements located upstream of the minimal TATA promoter. Stimulation of NFAT can therefore be monitored by measuring luciferase activity.

PD-1 expression was verified by flow cytometry and this cell line was functionally validated with Anti-PD-1 and Anti-PD-L1 neutralizing antibodies in co-culture assays.

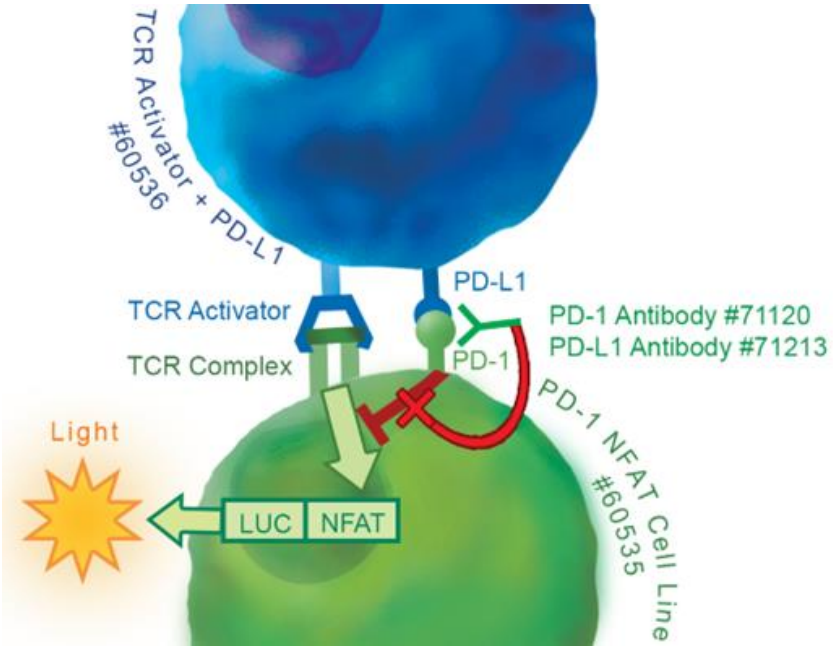


Figure 1: Illustration of the mechanism of action of PD-1 / NFAT Reporter Jurkat Recombinant Cell Line in a co-culture assay.

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. PD-1 ligands PD-L1 and PD-L2 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 immuno-therapy.

Application

- Screen for inhibitors of PD-1 or PD-L1 signaling in a cellular model.
- Characterize the biological activity of PD-1 and interaction with its ligands.

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
Jurkat (clone E6-1), human T lymphoblast, suspension

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 2	BPS Bioscience #60184
Growth Medium 2A	BPS Bioscience #60190

Materials Required for Cellular Assay

Name	Ordering Information
PD-L1 / TCR Activator CHO Cell Line	BPS Bioscience #60536
NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60621
Thaw Medium 3	BPS Bioscience #60186
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, 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 2 (BPS Bioscience #60184):

RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):

RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1 mg/ml of Geneticin, and 200 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):

RPMI1640 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 2.

   Note: 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 2.

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

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2, 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 reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2A**.**

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, in Growth Medium 2A**.** The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

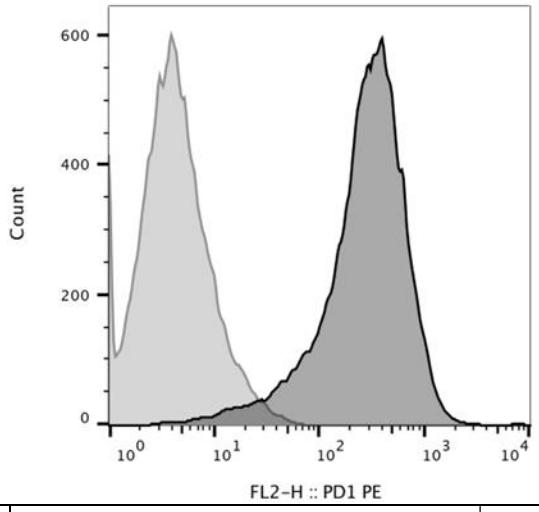
1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. 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.

3. 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.

Validation Data



	Samples	Cell Count
	NFAT Reporter Jurkat Cell Line	28,165
	PD-1/NFAT reporter Jurkat Cell Line	27,005

Figure 2. Cell surface expression analysis of PD-1 in PD-1/NFAT Reporter Jurkat Recombinant Cell Line by flow cytometry.
PD-1/NFAT Reporter Jurkat cells (dark grey) or control NFAT Reporter Jurkat cells (light grey) were stained with Anti-PD-1 Neutralizing Antibody, PE-labeled (BPS Bioscience #71290) and analyzed by flow cytometry. Y-axis represents the cell count. X-axis indicates PE intensity.

Cellular Assay Protocol

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 assay should include “Cell-Free Control”, "Untreated Control” and “Treated” conditions.
- The use of NFAT Luciferase Reporter Jurkat Cell Line as control is recommended.

A. Anti-PD-1 Neutralizing Antibody testing on PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured with the PD-L1/TCR Activator CHO 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.

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.

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

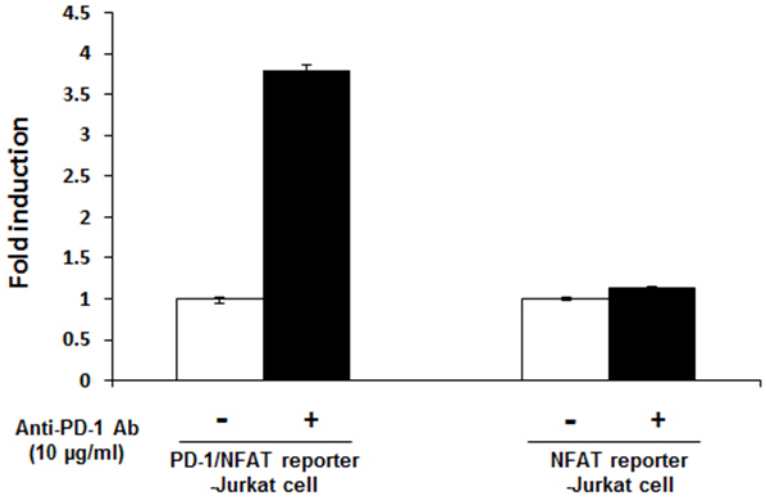


Figure 3. Effect of Anti-PD-1 Neutralizing Antibody on NFAT activation in the PD-1/NFAT Reporter Jurkat Recombinant Cell Line.
A co-culture assay was performed with the PD-L1/TCR-Activator CHO cell line as described in the protocol above. Addition of Anti-PD-1 Neutralizing Antibody increased NFAT-induced 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.

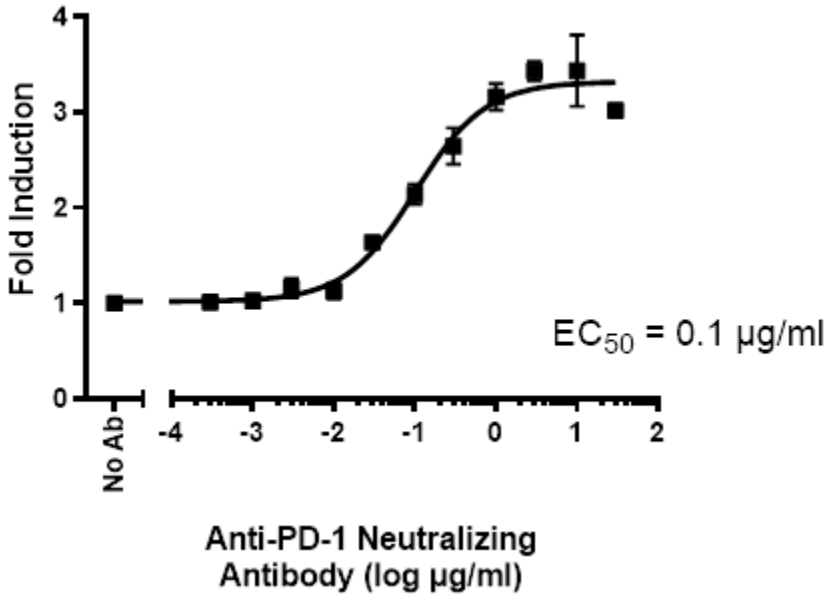


Figure 4. Dose-response curve of PD-1/NFAT Reporter Jurkat Recombinant Cell Line to Anti-PD-1 Neutralizing Antibody.
A co-culture assay was performed with the PD-L1/TCR-Activator CHO Cell Line as described in the protocol above, in the presence of increasing concentrations of Anti-PD-1 Neutralizing Antibody. 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 in the Jurkat cells.

B. Anti-PD-L1 Neutralizing Antibody testing on PD-1/NFAT Reporter Jurkat Recombinant Cell Line co-cultured with the PD-L1/TCR Activator CHO 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.

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.

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

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.

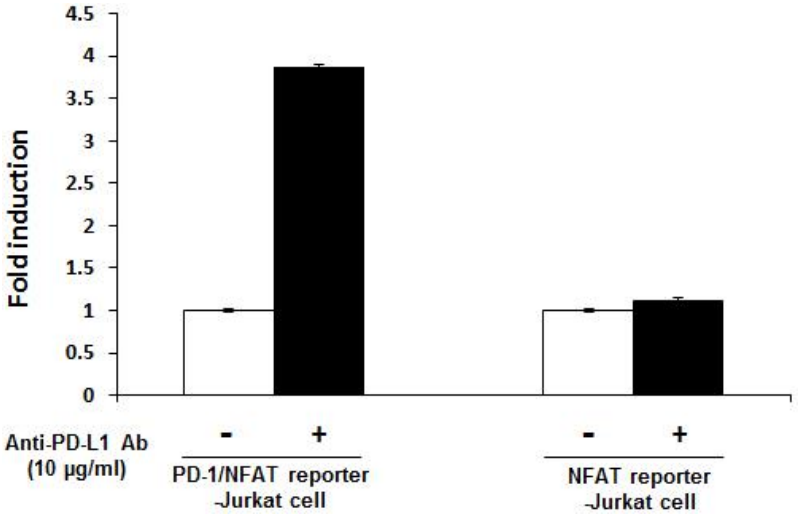


Figure 5. Effect of Anti-PD-L1 Neutralizing Antibody on NFAT activation in the PD-1/NFAT Reporter Jurkat Recombinant Cell Line.

A co-culture assay was performed with the PD-L1/TCR-Activator CHO Cell Line 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.

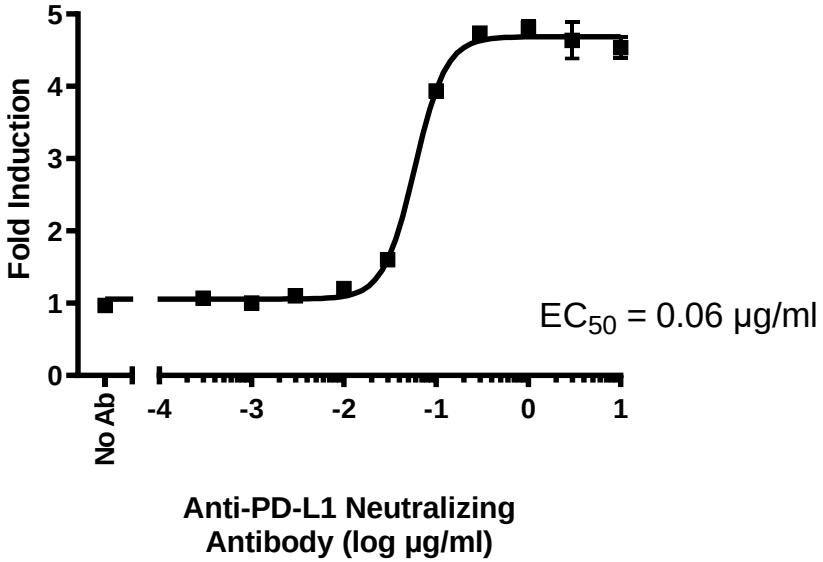


Figure 6. Dose response curve of PD-1/NFAT Reporter Jurkat Recombinant Cell Line to PD-L1 Neutralizing Antibody.

A co-culture assay was performed with the PD-L1/TCR-Activator CHO cell line 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 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-1 sequence (accession number NM_005018)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAA
FPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQF
QTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATI
VFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

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*
Anti-PD-1 Neutralizing Antibody, PE-labeled	71290	50 µg/100 µg
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/100 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	50 µg
PD-L1 (CD274), Fc fusion, Biotin-labeled (Human) HiP™	71105	50 µg

Version 011724