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Data Sheet

PD-1 / NFAT - Reporter - Jurkat Recombinant Cell Line

Catalog #: 60535

Product Description

Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of human PD-1 (Programmed Cell Death 1, PDCD1, SLEB2, CD279, GenBank Accession #NM_005018).

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.

Application

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

Format

Each vial contains 2 x 10^6 cells in 1 ml of 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) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 2 (BPS Cat. #60184): RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2A (BPS Cat. #60190): Thaw Medium 2 (BPS Cat. #60184), 1 mg/ml of Geneticin (Life Technologies #11811031), and 200 µg/ml of Hygromycin B (Hyclone #SV30070.01).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2A (Thaw Medium 2, Geneticin, and Hygromycin B).

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To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. This cell line tends to grow more slowly than parental Jurkat cells. After 24 hours of culture, add an additional 3 – 4 ml of growth medium without antibiotics. At first passage, switch to Growth Medium 2A **(contains Geneticin and Hygromycin B)**. Cells should be split before they reach $2x10^6$ cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than $0.2x10^6$ cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

Functional Validation and Assay Performance
Expression of human PD-1 in Jurkat cell line was confirmed by Western blotting.

The functionality of the cell line was validated using a PD-1:PD-L1 (or PD-L2) cell-based assay. In this assay, PD-1/NFAT Reporter/Jurkat T cells are used as effector cells; HEK293 or CHO cells over-expressing PD-L1 (or PD-L2) and an engineered T cell receptor (TCR) activator 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, PD1 and PD-L1 (or PD-L2) ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by anti-PD1 or anti-PD-L1 antibodies. PD1/PD-L1 neutralizing antibodies block PD1:PD-L1 interaction and promote T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter.

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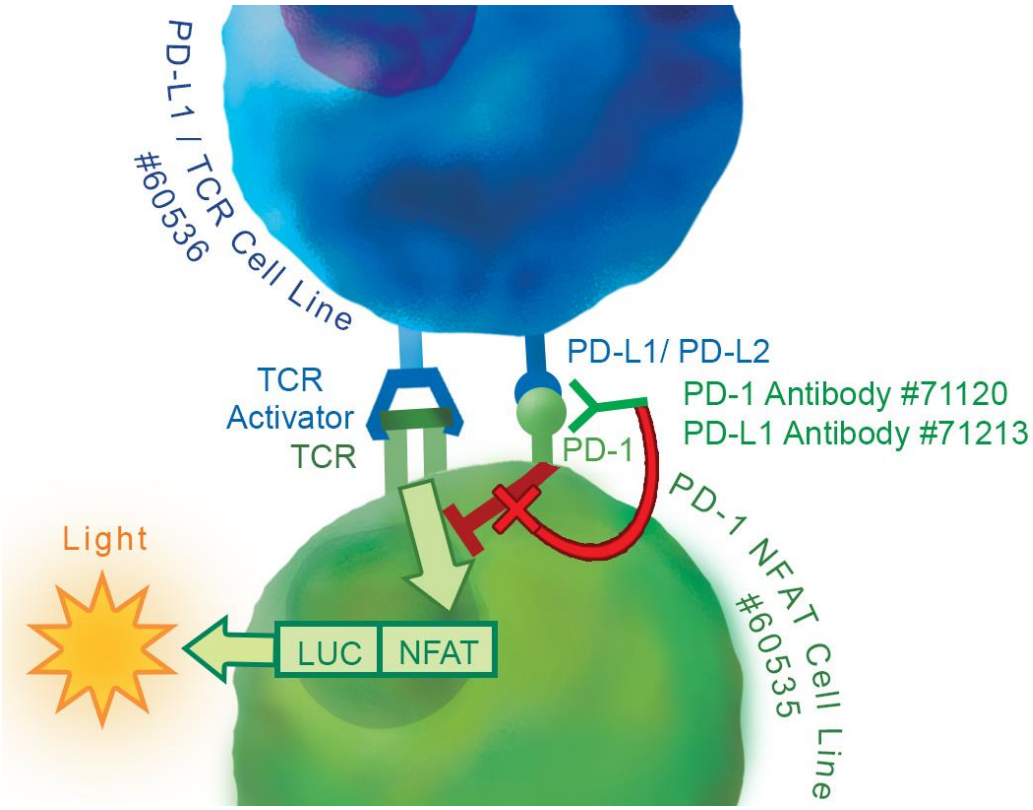
Assay Principle



Materials Required but Not Supplied

- TCR-activator PD-L1 CHO cell line (BPS Cat.#60536)
- Assay medium: Thaw Medium 2 (BPS Cat. #60184)
- Growth Medium 2A (BPS Cat. #60190)
- Anti-PD-1 neutralizing antibody: BPS bioscience #71120
- Anti-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).

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Protocol

1. Harvest TCR activator / PD-L1-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 Cat. #60186 (growth medium without Geneticin and Hygromycin B). Incubate cells at 37° in a CO2 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-PD-1 antibody or anti-PD-L1 antibody in assay medium (Thaw Medium 2, BPS Cat. #60184) (the concentration of antibody here is 2x of the final treatment concentration of antibody). Harvest the PD-1/NFAT-reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute cells to $4x10^5$ / ml in assay medium.

   To test anti-PD-1 antibody, preincubate the PD-1/NFAT Reporter- Jurkat cells ($4x10^5$ / ml) with diluted anti-PD-1 antibody (1:1 in volume) for 30 min. After incubation, remove the medium from TCR activator/PD-L1-CHO cells and add 100 µl of PD-1/NFAT reporter – Jurkat cells / anti-PD-1 antibody mixture to the wells. (Note: *Mix the PD-1/NFAT Reporter- Jurkat cells with antibody well before adding to TCR activator/PD-L1-CHO cells.*)

   To test the anti-PD-L1 antibody, remove the medium from TCR activator/PD-L1-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 ($4x10^5$ / ml) to the wells. (Note: *Mix the PD-1/NFAT Reporter- Jurkat cells well before adding to TCR activator/PD-L1-CHO cells.)*

   Final cell density of PD-1/NFAT Reporter- Jurkat cells is 2 $x10^4$ /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 CO2 incubator for 5 to 6 hours.

3. After ~5 to 6 hour incubation, perform 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 ~30 minutes. Measure luminescence using a luminometer.

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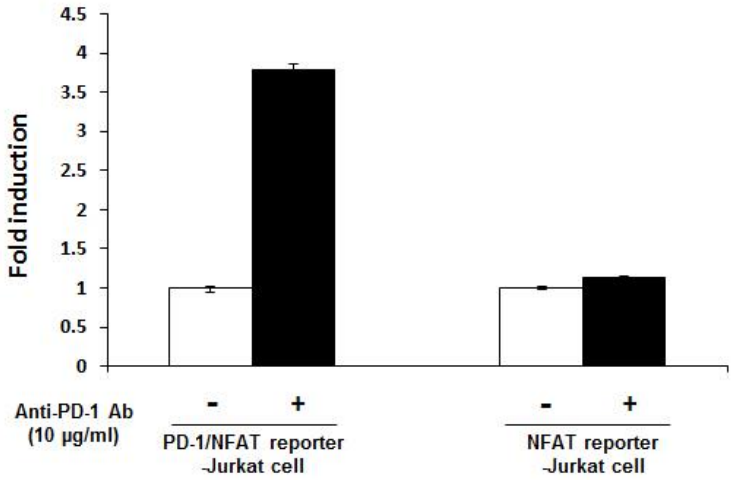
If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

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.

Figure 1. Cell Characterization Using a PD-1 Neutralizing Antibody. TCR activator/PD-L1-CHO cells were seeded in 96-well plate. The next day, TCR activator/PD-L1-CHO cells were incubated with anti-PD-1 neutralizing antibody (BPS Cat. #71120) and PD-1/NFAT Reporter-Jurkat cells (BPS Cat. #60535) (or control NFAT Reporter – Jurkat cells, BPS Cat. #60621). After incubation, ONE-Step™ Luciferase reagent (BPS Cat. #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.

A. Anti-PD-1 neutralizing antibody induced NFAT luciferase reporter activity in PD-1/NFAT Reporter-Jurkat cells, but not NFAT Reporter – Jurkat cells, co-cultured with TCR activator/PD-L1-CHO cells.



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B. Dose response of anti-PD-1 neutralizing antibody in PD-1/NFAT Reporter-Jurkat cells

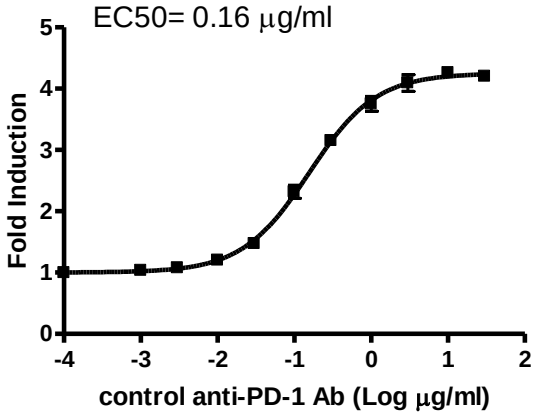

Figure 2. Cell Characterization Using a PD-L1 Neutralizing Antibody TCR activator/PD-L1-CHO cells were seeded in 96-well plate. The next day, TCR activator/PD-L1-CHO cells were incubated with anti-PD-L1 neutralizing antibody (BPS Cat. #71213) and PD-1/NFAT Reporter-Jurkat cells (BPS Cat. # 60535) (or control NFAT Reporter – Jurkat cells, BPS Cat. #60621). After incubation, ONE-Step™ Luciferase reagent (BPS Cat. #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.

A. Anti-PD-L1 neutralizing antibody induced NFAT luciferase reporter activity in PD-1/NFAT Reporter-Jurkat cells, but not NFAT Reporter – Jurkat cells, co-cultured with TCR activator / PD-L1 – CHO cells.

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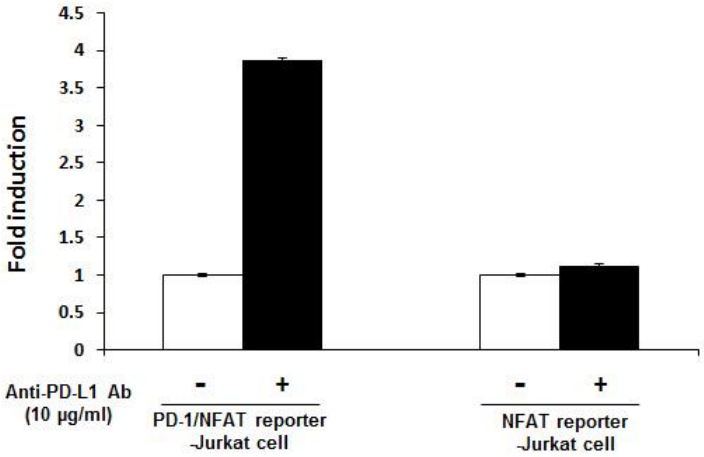

B. Dose response curve of anti-PD-L1 neutralizing antibody in PD-1/NFAT Reporter-Jurkat cells

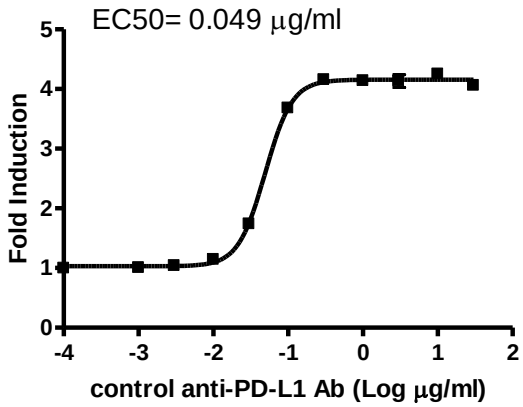

Figure 3. FACS analysis of cell surface expression of PD-1 in PD-1/NFAT Reporter-Jurkat cells.
PD-1/NFAT Reporter-Jurkat cells or control NFAT Reporter-Jurkat cells were stained with PE-labeled anti-PD-1 antibody (BPS Cat # 71290) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.

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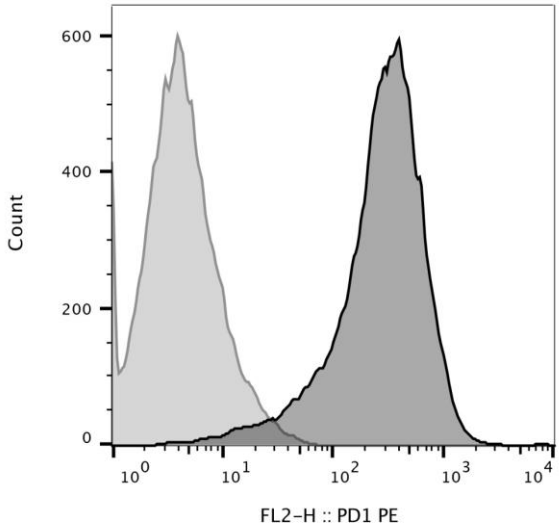

	Samples	Cell Count
	NFAT reporter-Jurkat	28165
	PD-1/NFAT-Jurkat	27005

Sequence

hPD-1 sequence (accession number NM_005018)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFV
LNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISL
APKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGLLGSLVLLVWVLAVIC
SRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSG
MGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

Related Products

Product	Cat. #	Size
NFAT Reporter – Jurkat cell line	60621	2 vials
TCR-activator CHO PD-L1 cell line	60536	2 vials
Anti-PD-1 neutralizing antibody	71120	100 µg
Anti-PD-L1 neutralizing antibody	71213	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Anti-PD-1 Antibody, PE-labeled	71290-1	50 µg
Anti-PD-1 Antibody, PE-labeled	71290-2	100 µg
Human PD-1 (CD279), Fc fusion	71106	100 µg
Human PD-1, FLAG-Avi-His-tag	71198	50 µg

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Human PD-L1 (CD274), Fc fusion	71104-1	50 µg
Human PD-L1 (CD274), Fc fusion	71104-2	100 µg
Human PD-L1 (CD274), FLAG-Avi-His tag	71183	50 µg
Human PD-L2 (CD273), Fc fusion	71107	100 µg
Human PD-1, Fc fusion, Biotin-labeled	71109	50 µg
Human PD-L1, Fc fusion, Biotin-labeled	71105	50 µg

Notes

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