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- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

Growth Arrested PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line
Catalog #: 79687
Size: 96 reactions

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). Note: These cells are unable to complete mitosis and are suitable for single use assays. For cells capable of reproducing, please use our PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line, #60535.

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.

The cell cycle control system acts like a timer, or a clock, that sets a fixed amount of time for the cell to spend in each phase of the cell cycle. The four major phases of the mammalian cell cycle are G1, S, G2 and M phases. Cell-cycle arrest means the cell enters quiescent stage, where the cell becomes a permanent cell and is no longer active in the process of cell division.

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 ~4 x 10^6 cells in 1 ml of FBS with 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, #MP0025) 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).

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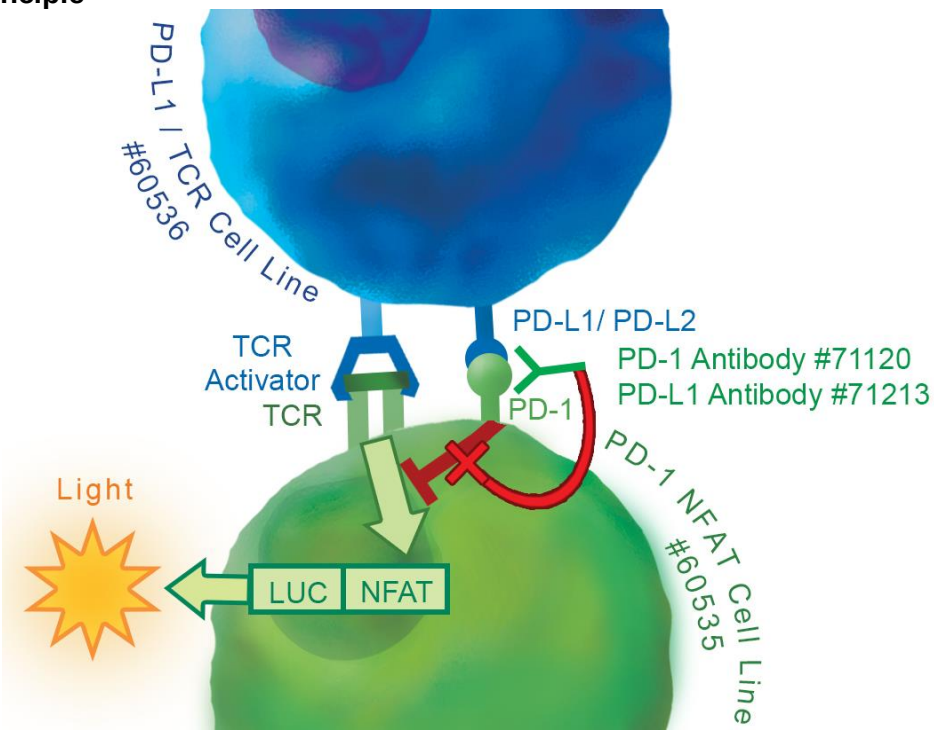
Cells should be grown at 37°C with 5% CO_2 using **Thaw Medium 2**.
To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2. Spin down the cells, remove supernatant and resuspend cells in 7 ml pre-warmed Thaw Medium 2. Immediately treat cells and seed into assay plates (see sample protocol below), and incubate at 37°C in a 5% CO_2 incubator. **These cells will not divide and will begin to die off around 96 hours after thaw.**

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

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.

Assay Principle



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Materials Required but Not Supplied

- PD-L1/ TCR-activator CHO cell line (BPS Bioscience, #60536)
  or PD-L2/ TCR-activator - CHO Recombinant Cell Line (BPS Bioscience, #79632)
- Assay medium: Thaw Medium 2 (BPS Bioscience, #60184)
- 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 Bioscience, #60186): Ham's F-12 medium (Hyclone, #SH30526.01) supplemented with 10% FBS (Life technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Sample 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 Bioscience #60186. Incubate cells at 37°C in a CO_2 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 Bioscience #60184); the concentration of antibody here should be 2x of the final treatment concentration of antibody. Quickly thaw the growth-arrested PD-1 Effector 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 assay medium. Spin down the cells at 1500 rpm, remove supernatant and re-suspend cells in 7 ml of pre-warmed assay medium (cell count of ~5.5 x 10^5 / ml).

   To test anti-PD-1 antibody, preincubate the PD-1/NFAT Reporter- Jurkat cells (~5.5 x 10^5 / 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 thoroughly 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 (~5.5 x 10^5 / ml) to the wells. (Note: *Mix the PD-1/NFAT Reporter- Jurkat cells thoroughly before adding to TCR activator/PD-L1-CHO cells.)*

   Final cell density of PD-1/NFAT Reporter- Jurkat cells is ~2.8 x 10^4 /well. Set up each treatment in at least triplicate.

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Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

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

3. After 6-hour incubation, perform luciferase assay using the ONE-Step™ luciferase assay system: Prepare ONE-Step reagent as recommended. Add 100 µl of ONE-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.
   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. Dose response curve of anti-PD-1 neutralizing antibody in PD-1:PD-L1 cell-based assay
HEK293 cells were transiently transfected with human PD-L1 and an engineered T cell receptor (TCR) activator. The next day, growth-arrested PD-1 Effector cells were pre-incubated with anti-PD-1 neutralizing antibody (BPS Bioscience #71120) for 30 minutes prior to co-culture with transfected HEK293 cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent 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.

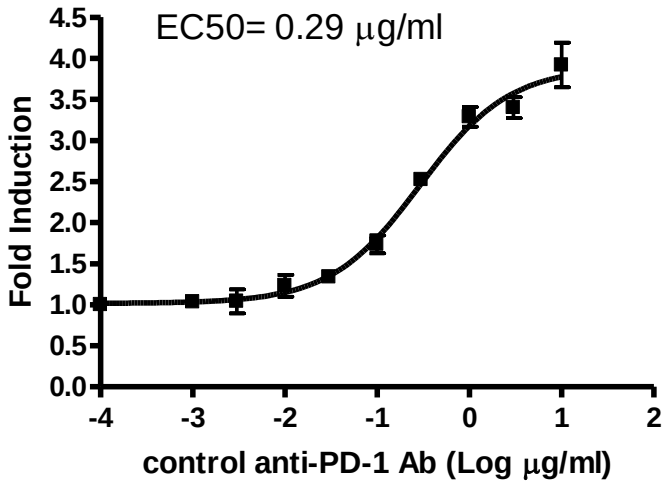

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Figure 2. Dose response curve of anti-PD-L1 neutralizing antibody in PD-1:PD-L1 cell-based assay

HEK293 cells were transiently transfected with human PD-L1 and an engineered T cell receptor (TCR) activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-L1 neutralizing antibody (BPS Bioscience #71213) for 30 minutes prior to co-culture with growth-arrested PD-1 effector cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent 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.

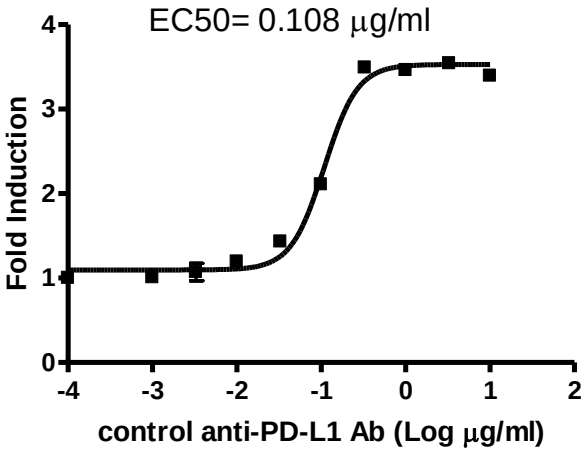

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Figure 3. Dose response curve of anti-PD-1 neutralizing antibody in PD-1:PD-L2 cell-based assay

HEK293 cells were transiently transfected with human PD-L2 and an engineered T cell receptor (TCR) activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-1 neutralizing antibody (BPS Bioscience #71120) for 30 minutes prior to co-culture with growth-arrested PD-1 effector cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent 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

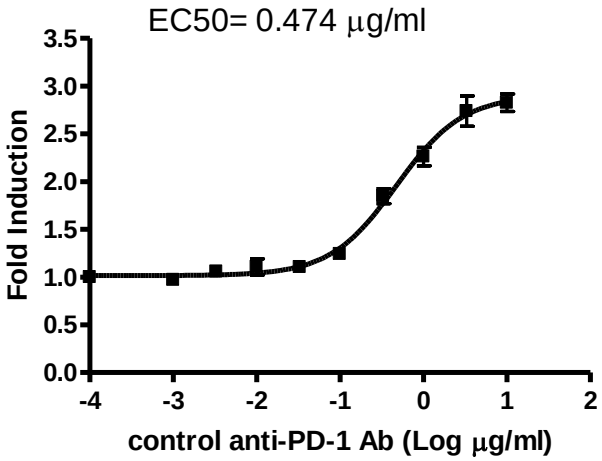

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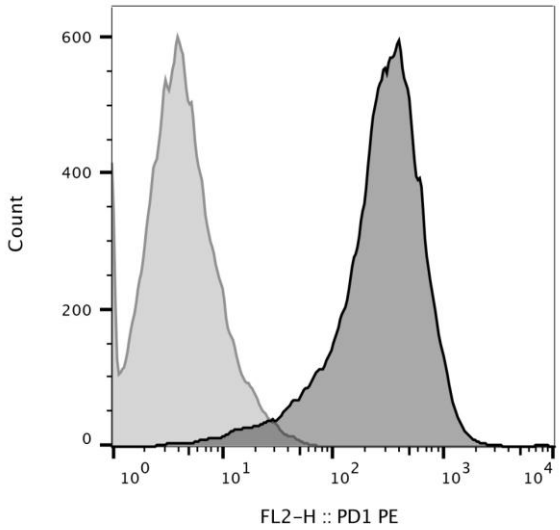
Figure 4: FACS analysis of PD-1/NFAT Reporter Jurkat cell line.
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line (dark grey) or control NFAT Reporter Jurkat Cell Line (BPS Bioscience, #60621, light grey), measured by FACS analysis using anti-PD-1 antibody (BPS Bioscience #71120).



	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

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Related Products

Product	Cat. #	Size
NFAT Reporter – Jurkat cell line	60621	2 vials
TCR-activator CHO PD-L1 cell line	60536	2 vials
TCR-activator CHO PD-L2 cell line	79632	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	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
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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