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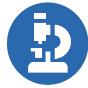 Laborgeräte & Service

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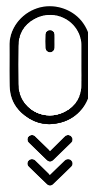

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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Woodchuck PD-1 / NFAT - Reporter - Jurkat Recombinant Cell Line
Catalog #: 79456

Product Description
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of woodchuck (groundhog, *Marmota monax*) PD-1 (Programmed Cell Death 1, PDCD1, SLEB2, CD279, GenBank Accession #HQ403652).

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 woodchuck PD-1 signaling in a cellular context
- Characterize the biological activity of woodchuck 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, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions
Thaw Medium 2 (BPS Bioscience, #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 Bioscience, #60190): Thaw Medium 2, 1 mg/ml of Geneticin (Thermo Fisher, #11811031), and 200 µg/ml of Hygromycin B (Thermo Fisher, #10687010).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2A.

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

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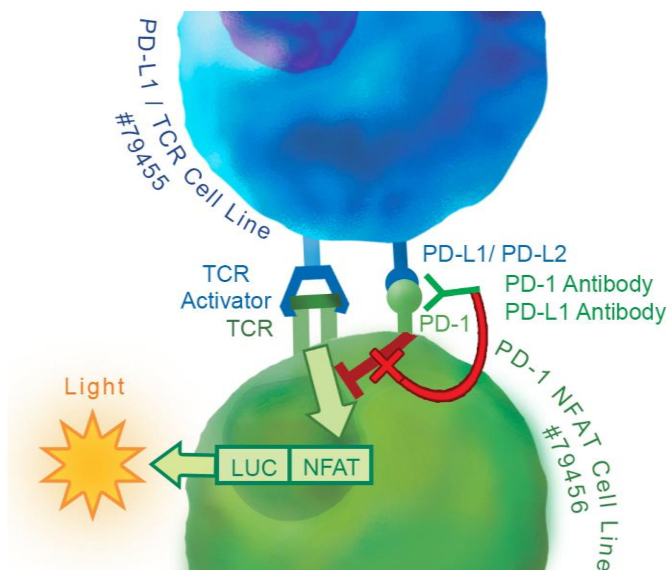
Medium 2 **(no Geneticin and Hygromycin B)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. This cell line tends to grow more slowly than parental Jurkat cells. After 24 hours of culture, add an additional 3 – 4 ml of growth medium without antibiotics. At first passage, switch 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 0.2×10^6 cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

Functional Validation and Assay Performance
Expression of woodchuck PD-1 in Jurkat cell line was confirmed by Flow Cytometry.

The functionality of the cell line was validated using a woodchuck PD-1:PD-L1 cell-based assay. In this assay, woodchuck PD-1/NFAT Reporter/Jurkat T cells are used as effector cells; HEK293 or CHO cells over-expressing woodchuck PD-L1 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 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



Protocol 1: Woodchuck PD-1/PD-L1 cell-based assay using the woodchuck PD-1/NFAT Reporter-Jurkat cells and HEK293 cells transiently transfected with woodchuck PD-L1

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San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Materials Required but Not Supplied

- HEK293 cell and its growth medium
- TCR Activator/Woodchuck PD-L1 Mammalian Expression Kit (BPS Bioscience #79455)
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Life technologies #11668027). However, other transfection reagents work equally well.]
- Opti-MEM I Reduced Serum Medium (life technologies #31985-062)
- Assay medium: Thaw Medium 2 (BPS Bioscience #60184)
- 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

1. One day before transfection, seed HEK293 cells at a density of 35,000 cells per well in 100 µl of Growth Medium 2A so that cells will be 90% confluent at the time of transfection.

2. Next day, transfect 1 µl of Expression vectors for engineered TCR activator and woodchuck PD-L1 into cells following the manufacturer's protocol.

3. One day after transfection, prepare serial dilution of antibody in assay medium (the concentration of antibody here is 2x of the final treatment concentration of antibody). Harvest the woodchuck PD-1/NFAT-reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute cells to 4×10^5 / ml in assay medium.

   To test anti-PD-1 antibody, preincubate the woodchuck PD-1/NFAT Reporter- Jurkat cells (4×10^5 /ml) with diluted anti-PD-1 antibody (1:1 in volume) for 15-30 min. After incubation, remove the medium from transfected HEK293 cells and add 100 µl of woodchuck PD-1/NFAT reporter – Jurkat cells / anti-PD-1 antibody mixture to the wells. (Note: *Mix the woodchuck PD-1/NFAT Reporter- Jurkat cells with antibody thoroughly immediately before adding to transfected HEK293 cells.)*

   To test the anti-PD-L1 antibody, remove the medium from transfected HEK293 cells, add 50 µl of diluted anti-PD-L1 antibody to the wells and incubate for 30 min. After incubation, add 50 µl of woodchuck PD-1/NFAT Reporter- Jurkat cells (4×10^5 / ml) to the wells. (Note: *Mix the woodchuck PD-1/NFAT Reporter- Jurkat cells thoroughly immediately before adding to the transfected HEK293 cells.)*

   Final cell density of woodchuck PD-1/NFAT Reporter- Jurkat cells is 2×10^4 /well. Set up each treatment in at least triplicate. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

4. After ~16 hours, measure the luciferase expression using the ONE-Step luciferase assay system, following recommended assay protocol. Add 100 µl of ONE-Step Luciferase

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reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer.

5. 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 stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. The reporter activity from woodchuck PD-1/NFAT reporter Jurkat cells is decreased when co-cultured with HEK293 cells transiently transfected with woodchuck PD-L1

HEK293 cells were transiently transfected with the genes for woodchuck PD-L1 and an engineered T cell receptor (TCR) activator. The next day, woodchuck PD-1/NFAT Reporter-Jurkat cells (Figure 1B) or control NFAT Reporter – Jurkat cells (Figure 1A) were co-cultured with transfected HEK293 cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure NFAT activity.

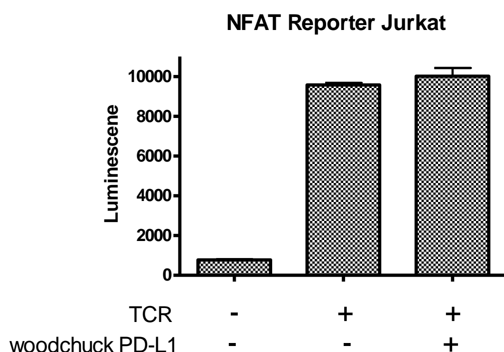

Figure 1A

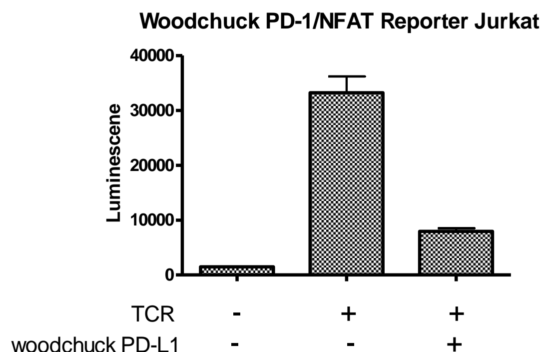

Figure 1B

Protocol 2: Woodchuck PD-1/PD-L1 cell-based assay using woodchuck PD-1/NFAT Reporter-Jurkat cells and woodchuck PD-L1/ TCR Activator CHO cells

Materials Required but Not Supplied

- Woodchuck PD-L1/TCR activator CHO cell line (BPS Bioscience #79457)
- Assay medium: Thaw Medium 2 (BPS Bioscience #60184)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience #60690)
- Luminometer

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1. Harvest TCR activator / woodchuck 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 2. Incubate cells at 37°C in a CO_2 incubator overnight. Cells should reach ~80% confluency by the next day (do not allow cells to reach confluency in this step).

2. Next day, prepare serial dilution of anti-PD-1 antibody or anti-PD-L1 antibody in assay medium (the concentration of antibody here is 2x of the final treatment concentration of antibody). Harvest the woodchuck PD-1/NFAT-reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute cells to 4 x 10^5 / ml in assay medium.

   To test anti-PD-1 antibody, preincubate the woodchuck PD-1/NFAT Reporter- Jurkat cells (4 x 10^5 /ml) with diluted anti-PD-1 antibody (1:1 in volume) for 30 min. After incubation, remove the medium from woodchuck PD-L1/ TCR activator-CHO cells and add 100 µl of woodchuck PD-1/NFAT reporter – Jurkat cells / anti-PD-1 antibody mixture to the wells. (Note: *Mix the woodchuck PD-1/NFAT Reporter- Jurkat cells with antibody thoroughly immediately before adding to woodchuck PD-L1/TCR activator-CHO cells.*)

   To test the anti-PD-L1 antibody, remove the medium from woodchuck PD-L/ TCR activator/-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 woodchuck PD-1/NFAT Reporter- Jurkat cells (4 x 10^5 /ml) to the wells. (Note: *Mix the woodchuck PD-1/NFAT Reporter- Jurkat cells thoroughly immediately before adding to woodchuck PD-L1/TCR activator-CHO cells.)*

   Final cell density of woodchuck PD-1/NFAT Reporter- Jurkat cells is 2 x 10^4 /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°C in a CO_2 incubator for 5 to 6 hours.

3. After ~5 to 6 hour incubation, perform luciferase assay using the ONE-Step luciferase assay system, following the recommended protocol. Add 100 µl of One-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.

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 2. Woodchuck PD-1/PD-L1 cell-based assay using the woodchuck PD-1/NFAT Reporter-Jurkat cells and woodchuck PD-L1/ TCR Activator CHO cells.TCR activator/woodchuck PD-L1-CHO cells were seeded in 96-well plate. The next day, woodchuck PD-L1/TCR activator-CHO cells were incubated with anti-PD-L1 neutralizing antibody and woodchuck PD-1/NFAT Reporter-Jurkat cells (BPS Bioscience 79456; Figure 2B) or control NFAT Reporter – Jurkat cells (BPS Bioscience #60621; Figure 2A). After incubation, ONE-

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Email: info@bpsbioscience.com

Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure NFAT activity. As shown in Figure 2, anti-mouse PD-L1 (Fisher Scientific #50-146-65, clone#MIH5) and anti-woodchuck PD-L1 neutralizing antibodies can block the woodchuck PD-1/PD-L1 interaction while anti-human PD-L1 (BPS Bioscience #71213) cannot.

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.

Figure 2A

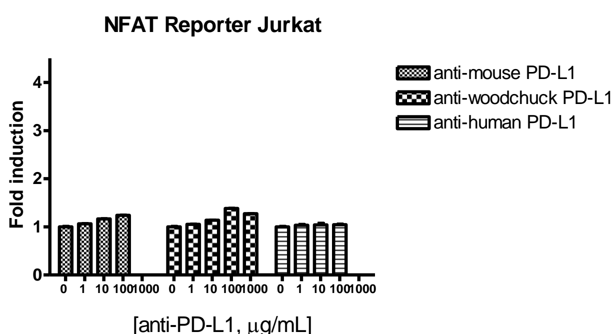

Figure 2B

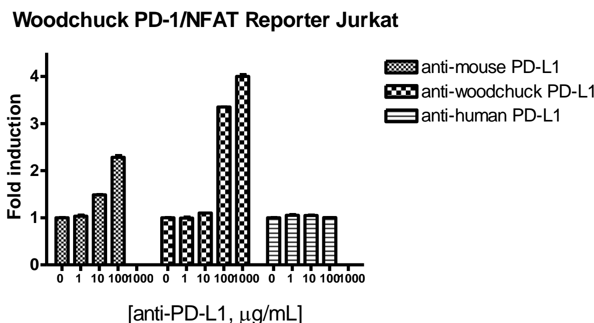

Figure 3. FACS analysis of cell surface expression of woodchuck PD-1 in PD-1/NFAT Reporter-Jurkat cells.

Woodchuck PD-1/NFAT Reporter-Jurkat cells (blue) or control NFAT Reporter-Jurkat cells (pink) were stained with PE-labeled anti-PD-1 antibody (ebioscience, CAT#14-9989-82; Clone#J116) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.

BPS Bioscience
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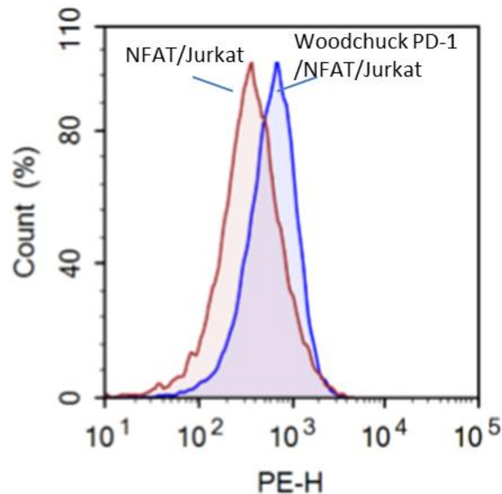

Sequence

Woodchuck PD-1 sequence (accession number HQ403652)

MQGRWVTWLLAWAVLQLGWRPGWLLESSNRPSSLFSCSPTQLTVQEGANATFTCSFSNWSEHLV
LNWYRLSPSKQNIKLASFRSGLSEPGRDPRFRVTQLPSRLDFHMSVISAQRSDSGLYLCGAISL
SSKVQIQETTAAELRVTDRVLESPTLEPLPAHPRPSPRPAGQLPGLVVGVTSVLVGVPVLLLLA
WVLATTCSTALPDAGGARSKEQPLEEASEVPVSTLDYGELDFQWRERTPTPEPPASCIHTEYAT
IVFPSSPGRRGSADSAQGPQPLRPEDGHCSWPL

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Related Products

Product	Cat. #	Size
NFAT Reporter – Jurkat cell line	60621	2 vials
Woodchuck PD-L1/TCR-activatorCHO cell line	79457	2 vials
Woodchuck PD-L1 /TCR Activator Mammalian Expression Kit	79455	500 rxns
PD-1/NFAT Reporter-Jurkat cell line	60535	2 vials
PD-L1/TCR Activator -CHO recombinant cell line	60536	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Woodchuck PD-1, Fc fusion	79314	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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