6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data Sheet

Woodchuck PD-L1 / TCR activator - CHO Recombinant Cell line
Cat. #: 79457

Product Description
Recombinant CHO-K1 cells constitutively expressing woodchuck (groundhog, *Marmota monax*) PD-L1 (Programmed Cell Death 1 Ligand 1, CD274**,** B7 homolog 1 (B7- H1), GenBank accession # HQ403651) and an engineered T cell receptor (TCR) activator.

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.

Applications
- Screen for activators or inhibitors of PD-1 signaling in a cellular context
- Screen PD-L1 antibodies for binding affinity
- Characterize the biological activity of PD-1 interactions with PD-L1

Format
Each vial contains ~2.5 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 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 B to ensure recombinant expression.

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3A. PD-L1 /TCR activator – CHO cells should exhibit a typical cell division time of ~24 hours.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 3 **(no Geneticin and Hygromycin B)**, spin down cells, and re-suspend cells in pre-warmed Thaw Medium 3 **(no Geneticin and Hygromycin B)**. Transfer re-suspended cells to a T25 flask and culture in 37°C CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 **(no Geneticin and Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch to Growth Medium 3A **(contains Geneticin and Hygromycin B)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 3A transfer to a tube, spin down cells, re-suspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 twice a week.

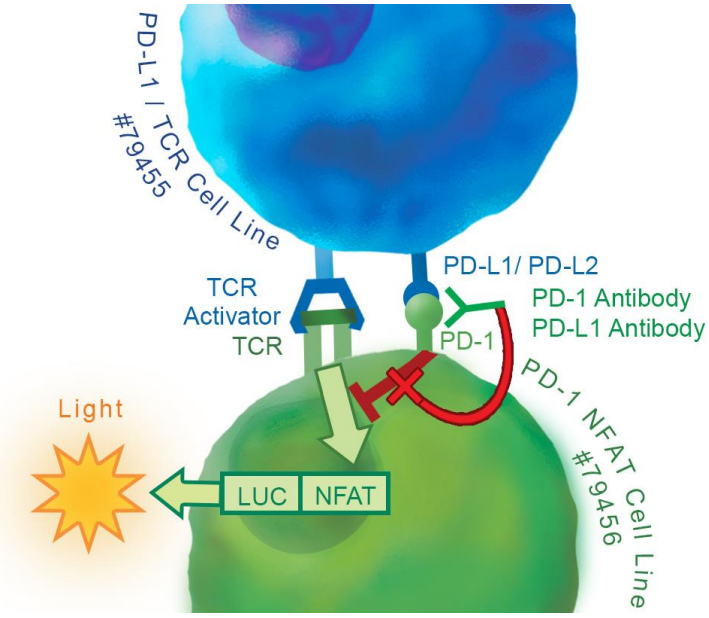
To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 3 **(no Geneticin or Hygromycin B)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

Functional Validation and Assay Performance
Expression of woodchuck PD-L1 in CHO-K1 cells was confirmed by FACS.

The functionality of the cell line was validated using a woodchuck PD-1:PD-L1 cell-based assay. In this assay, Jurkat T cells expressing NFAT reporter with constitutive expression of woodchuck PD-1 (woodchuck PD-1/NFAT Reporter/Jurkat, BPS Bioscience #79456) are used as effector cells; woodchuck PD-L1/TCR activator- CHO cells 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.

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Assay Principle



Materials Required but Not Supplied

- Woodchuck PD-1/ NFAT reporter-Jurkat cell line (BPS Bioscience #79456)
- Assay medium: RPMI1640 + 10% FBS + 1% Penicillin/Streptomycin (Thaw Medium 2, BPS Bioscience #60184)
- Thaw Medium 3 (BPS Bioscience #60186)
- Growth Medium 3B (BPS Bioscience #60188)
- 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
- Anti-woodchuck PD-1 or PD-L1 neutralizing antibodies. We have successfully used anti-mouse PD-L1 antibody (Fisher Scientific #50-146-65, clone #MIH5).

Protocol

1. Harvest woodchuck PD-L1/ TCR activator -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. Incubate cells at 37°C in a CO_2 incubator for overnight. Cells should reach ~80% confluency by the next day (do not allow cells to reach confluency at this step).

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

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 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-L1/ 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 (4x10^5 / 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 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 1. 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. Woodchuck PD-L1/ TCR activator-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 1B) or control NFAT Reporter – Jurkat cells (BPS Bioscience #60621; Figure 1A). After incubation, ONE-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

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

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 1A

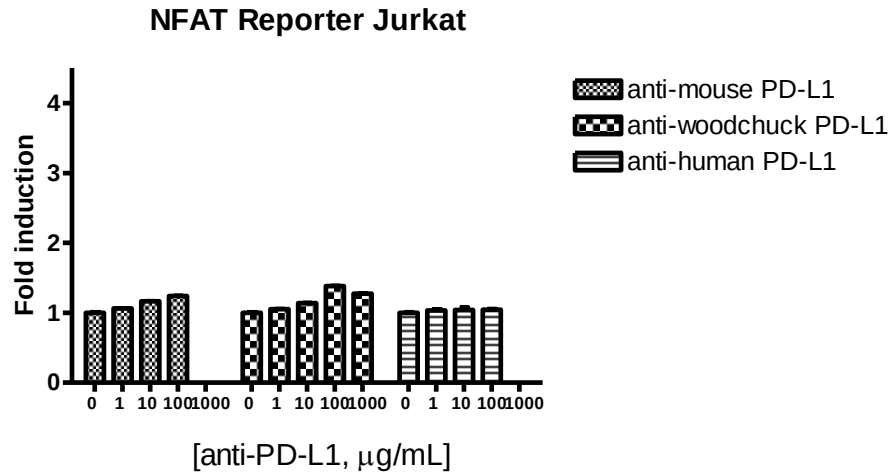
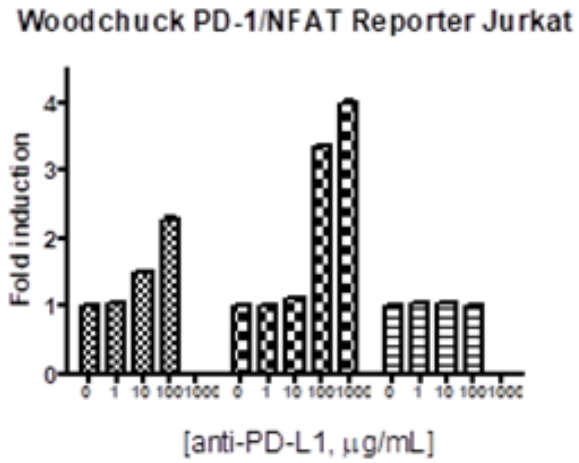
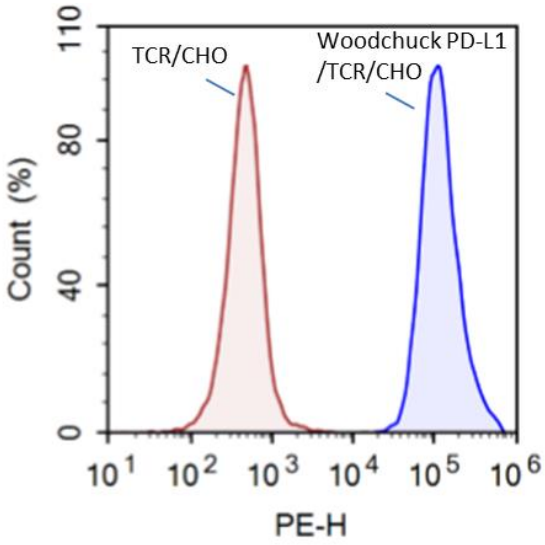


Figure 1B



6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Figure 2. FACS analysis of cell surface expression of PD-L1 in woodchuck PD-L1/ TCR Activator-CHO cells. Woodchuck PD-L1/ TCR activator-CHO (blue) or parental CHO-K1 cells (pink) were stained with PE-labeled anti-PD-L1 antibody (Fisher Scientific #50-112-9385, clone MIH5) and analyzed by FACS. Y-axis shows the cell count. X-axis shows the intensity of PE fluorescence.



Sequence

Woodchuck PD-L1 sequence (accession number HQ403651)

MRMFNVFIFTSFCHLLNAFSITVPKDLYVVEYGSNVTIECKFPVEKQLDLGSLVVYWGKEDEEI
IQFVNGKEDLKVQHSSYRQRAWLLKDQLYQGNAVLQITNVKLQDAGVYCCMISYGGADYKWITL
KVNAPYREISQRISMDPVTSEYELTCQAEGYPEAEVIWTSSDHQILSAKTTITKSQREEKFFNV
TSTLRINTTANEIFYCTFKRLSFTENNTAELVIPEPSTLLQRTHFVKLGAVLFCFGAALTILFC
LRKNVRMLDVENGGIQDINSRKQNDTQFEET

6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Related Products

Product	Cat. #
Woodchuck PD-1/NFAT Reporter-Jurkat cell line	79456
PD-1/NFAT Reporter-Jurkat cell line	60535
NFAT Reporter – Jurkat cell line	60621
TCR activator-CHO cell line	60539
Woodchuck PD-L1 /TCR activator expression kit	79455
ONE-Step™ Luciferase Assay System	60690-1
ONE-Step™ Luciferase Assay System	60690-2
Woodchuck PD-1, Fc fusion	79314
Human PD-1 (CD279), Fc fusion	71106
Human PD-1, FLAG-Avi-His-tag	71198
Human PD-L1 (CD274), Fc fusion	71104
Human PD-L1 (CD274), FLAG-Avi-His tag	71183
Human PD-L2 (CD273), Fc fusion	71107
Human PD-1, Fc fusion, Biotin-labeled	71109
Human PD-L1, Fc fusion, Biotin-labeled	71105

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