6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Woodchuck PD-L1 / TCR Activator Mammalian Expression Kit
Catalog #: 79455

Product Description
The recombinant expression vectors are designed to express human engineered T cell receptor (TCR) activator and woodchuck (groundhog, *Marmota monax*) PD-L1 (GenBank Accession #HQ403651) in mammalian cells. The transfected cells can be used in conjunction with woodchuck PD-1/NFAT Reporter/Jurkat T cells (BPS #79456) to study the interactions of PD-1 with PD-L1 ligand in a cellular context and screen for modulators of this signaling pathway.

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

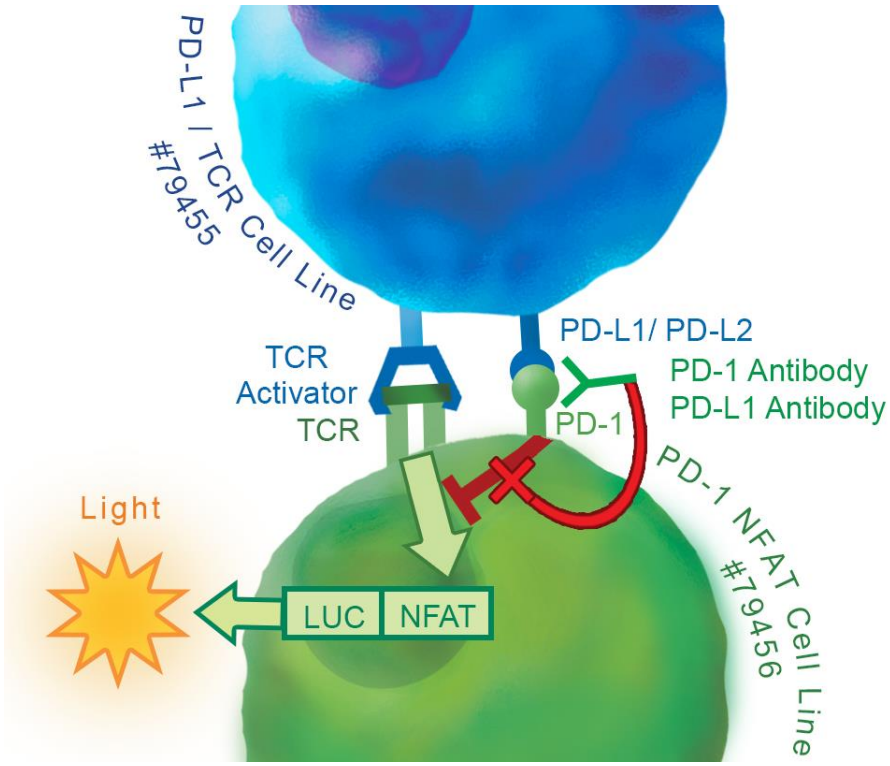
Components

Component	Specification	Amount	Storage
TCR activator + Woodchuck PD-L1 (Component A)	Expression vectors constitutively expressing TCR activator and woodchuck PD-L1	500 µl (100 ng DNA/µl)	-20°C
TCR activator (Component B)	Expression vector constitutively expressing TCR activator	500 µl (100 ng DNA/µl)	-20°C

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Functional Validation and Assay Performance

In this assay, woodchuck PD-1/NFAT Reporter/Jurkat T cells are used as effector cells; HEK293 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.



6044 Cornerstone Court W, Ste E
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 or other cell lines
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (life technologies, #11668027). However, other transfection reagents work equally well.]
- Woodchuck PD-1/NFAT Reporter Jurkat T cells (BPS Bioscience #79456)
- Opti-MEM I Reduced Serum Medium (life technologies #31985-062)
- Assay medium: RPMI1640 + 10% FBS + 1% Penicillin/Streptomycin
- 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. One day before transfection, seed HEK293 cells at a density of 35,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection. Leave a few cell-free wells for use as a background control for luminescence.

2. Next day, transfect 1 µl of the expression vectors for TCR activator and woodchuck PD-L1 (component A) or the control expression vector for only TCR activator (component B) into cells following the manufacturer's protocol.

3. One day after transfection, preincubate the corresponding cell line with the appropriate antibody prior to co-culturing the woodchuck PD-1/NFAT Reporter-Jurkat cells and the transfected HEK293 cells. Perform all assays in at least triplicate.

   To test the anti-PD-1 antibody, dilute the antibody in assay medium, remove the medium from the woodchuck PD-1/NFAT Reporter- Jurkat cells, and preincubate the anti-PD-1 antibody with woodchuck PD-1/NFAT Reporter- Jurkat cells for 30 minutes, then add the woodchuck PD-1/NFAT Reporter- Jurkat cells to the transfected HEK293 cells.
   In our lab, we make serial dilutions of antibody at 2x the final treatment concentration. Woodchuck PD-1/NFAT Reporter- Jurkat cells (4 x 10^5 / ml) are incubated with diluted anti-PD-1 antibody (1:1 in volume) for 30 min. After incubation, remove the medium from TCR activator/woodchuck PD-L1-CHO cells and add 100 µl of woodchuck PD-1/NFAT reporter – Jurkat cells / anti-PD-1 antibody mixture to the wells. Be sure to 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, dilute the antibody in assay medium, remove the medium from the transfected HEK293, and preincubate the anti-PD-L1 antibody with

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

transfected HEK293 for 30 min, then add the woodchuck PD-1/NFAT Reporter- Jurkat to transfected HEK293.

In our lab, we make serial dilutions of antibody at 2x the final treatment concentration. Remove the medium from woodchuck PD-L1/ TCR activator-CHO cells and add 50 µl of 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. Be sure to mix the woodchuck PD-1/NFAT Reporter- Jurkat cells with antibody thoroughly immediately before adding to TCR activator/woodchuck PD-L1-CHO cells.

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 the recommended protocol. Add 100 µl of One-Step Luciferase 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.

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

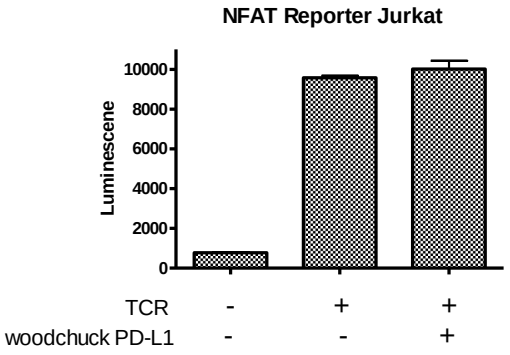


Figure 1A

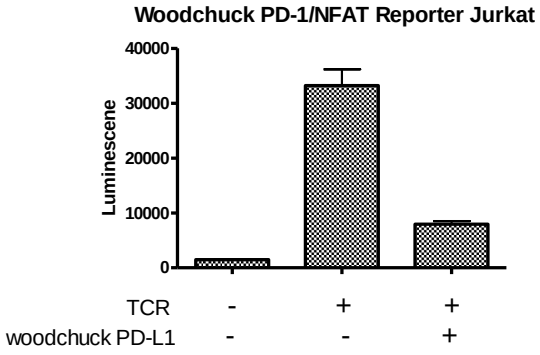


Figure 1B

Related Products

Product	**Cat. #**	**Size**
Woodchuck PD-1 / NFAT - Reporter - Jurkat Recombinant Cell Line	79456	2 vials
TCR-activator/ woodchuck PD-L1 CHO cell line	79457	2 vials
Woodchuck PD-1, Fc fusion	79314	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
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

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.
To place your order, please contact us by Phone **1.858.829.3082** Fax **1.858.481.8694**
Or you can Email us at: **info@bpsbioscience.com**
Please visit our website at: **www.bpsbioscience.com**