



6042 Cornerstone Court W, Ste B  
San Diego, CA 92121  
Tel: 1.858.202.1401  
Fax: 1.858.481.8694  
Email: info@bpsbioscience.com

## Data Sheet

### Mouse PD-L1/TCR Activator Mammalian Expression Kit Catalog #: 79778 500 Reactions

#### Product Description

The recombinant expression vectors are designed to express human engineered T cell receptor (TCR) activator and mouse PD-L1 (GenBank Accession #NM\_021893) in mammalian cells. The transfected cells can be used in conjunction with mouse PD-1/NFAT Reporter/Jurkat T cells (BPS Bioscience #79762) to study the interactions of PD-1 with PD-L1 ligand in a cellular context and to 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 mouse PD-1 signaling in a cellular context
- Characterize the biological activity of mouse PD-1 and its interactions with ligands

#### Components

| Component  | Specification  | Amount                    | Storage |
|--|--|---------------------------|---------|
| <b>TCR activator<br/>+<br/>Mouse PD-L1<br/>(Component A)</b> | Expression vectors constitutively expressing TCR activator and mouse PD-L1 | 500 µl<br>(100 ng DNA/µl) | -20°C   |
| <b>TCR activator<br/>(Component B)</b>                       | Expression vector constitutively expressing TCR activator                  | 500 µl<br>(100 ng DNA/µl) | -20°C   |

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

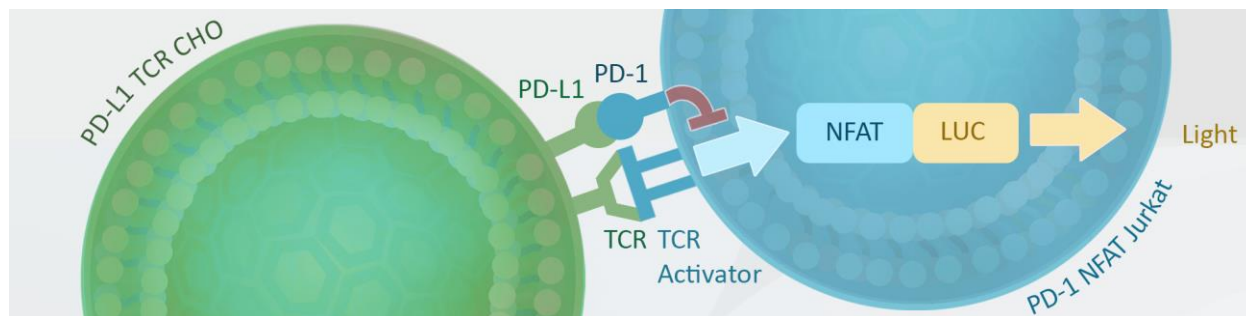
Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)

## Functional Validation and Assay Performance

In this assay, mouse PD-1/NFAT Reporter/Jurkat T cells are used as effector cells; HEK293 cells transiently transfected with the PD-L1 vector over-express mouse PD-L1 and an engineered T cell receptor (TCR) activator and 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. Mouse 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



## 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.]
- Mouse PD-1/NFAT Reporter Jurkat T cells (BPS Bioscience #79762)
- Opti-MEM I Reduced Serum Medium (life technologies #31985-062)
- Thaw Medium 2: RPMI1640 + 10% FBS + 1% Penicillin/Streptomycin (BPS Bioscience #60184)
- Anti-mouse PD-1 neutralizing antibody: Bioxcell #BP0273, clone#29F.1A12
- Anti-mouse PD-L1 neutralizing antibody: Fisher Scientific #50-146-65, clone#MIH5
- 96-well tissue culture-treated white clear-bottom assay plate
- (optional) NFAT Reporter (Luc) – Jurkat Recombinant Cell Line (BPS Bioscience #60621) as negative control for Mouse PD-1/NFAT Reporter Jurkat T cells (BPS Bioscience #79762)
- ONE-Step™ luciferase assay system (BPS Bioscience #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)



6042 Cornerstone Court W, Ste B  
San Diego, CA 92121  
**Tel:** 1.858.202.1401  
**Fax:** 1.858.481.8694  
**Email:** [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

## Protocol

1. One day before transfection, seed HEK293 cells at a density of 35,000 cells per well in 100  $\mu$ l of growth medium so that cells will be 90% confluent at the time of transfection.
2. Next day, transfect 1  $\mu$ l of the expression vectors for TCR activator and mouse 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 mouse PD-1/NFAT Reporter-Jurkat cells and the transfected HEK293 cells.

**To test the anti-mouse PD-1 antibody**, dilute the antibody in Thaw Medium 2, preincubate the mouse PD-1/NFAT Reporter- Jurkat cells ( $4 \times 10^5$  / ml) with diluted anti-PD-1 antibody (1:1 in volume) for 30 min. After incubation, remove the medium from the transfected HEK293 cells and add 100  $\mu$ l of mouse PD-1/NFAT reporter – Jurkat cells / anti-mouse PD-1 antibody mixture to the wells. (Note: *Mix the PD-1/NFAT Reporter- Jurkat cells with antibody well before adding to the transfected HEK293 cells.*)

**To test the anti-mouse PD-L1 antibody**, dilute the antibody in Thaw Medium 2, remove the medium from the transfected HEK293, and preincubate the anti-PD-L1 antibody with transfected HEK293 for 30 min, then add the mouse PD-1/NFAT Reporter- Jurkat to transfected HEK293.

Final cell density of mouse PD-1/NFAT Reporter- Jurkat cells is  $2 \times 10^4$  /well. Set up each treatment in at least triplicate

4. After ~16 hours, measure the luciferase expression using the ONE-Step luciferase assay system: Add 100  $\mu$ l 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.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

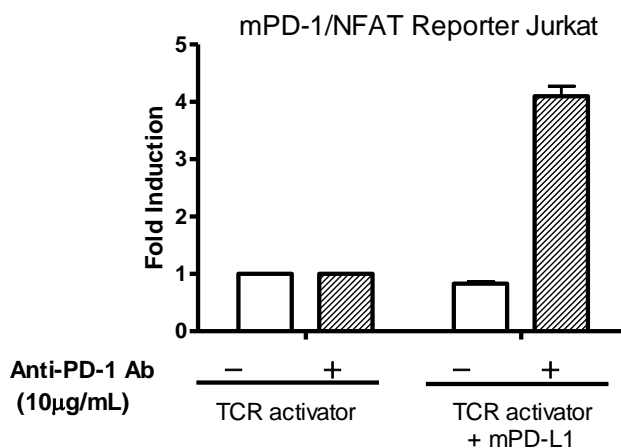
Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)

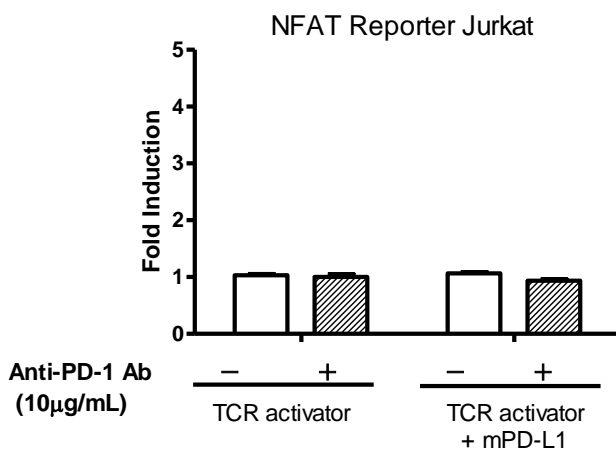
**Figure 1. Characterization of biological activity of anti-mouse PD-1 neutralizing antibody in PD-1/PD-L1 cell-based assay using the mouse PD-1/NFAT Reporter-Jurkat cells.**

HEK293 cells were transiently transfected with the vectors for mouse PD-L1 and the TCR activator. The next day, mouse PD-1/NFAT Reporter-Jurkat cells (or control NFAT Reporter – Jurkat cells) were pre-incubated with anti-mouse PD-1 neutralizing antibody (Bioxcell #BP0273) for 30 minutes prior to co-culture 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.

- A.** Anti-mouse PD-1 neutralizing antibody induced NFAT luciferase reporter activity in mouse PD-1/NFAT Reporter-Jurkat cells co-cultured with HEK293 cells overexpressing mouse PD-L1 and TCR activator.

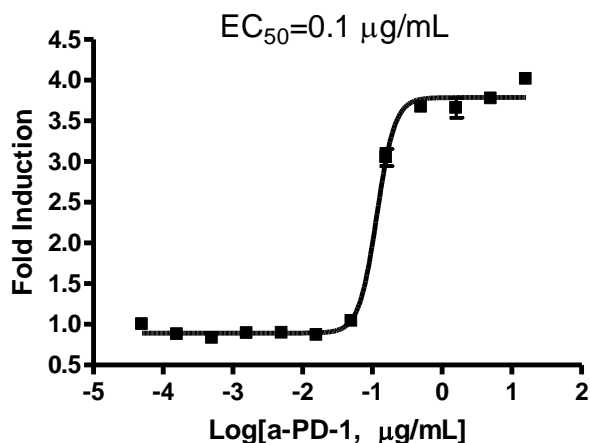


- B.** Anti-PD-1 neutralizing antibody had no effect on NFAT luciferase reporter activity in control NFAT Reporter-Jurkat cells (BPS Bioscience #60621) co-cultured with HEK293 cells overexpressing mouse PD-L1 and TCR activator.



OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.  
 To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**  
 Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)  
 Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)

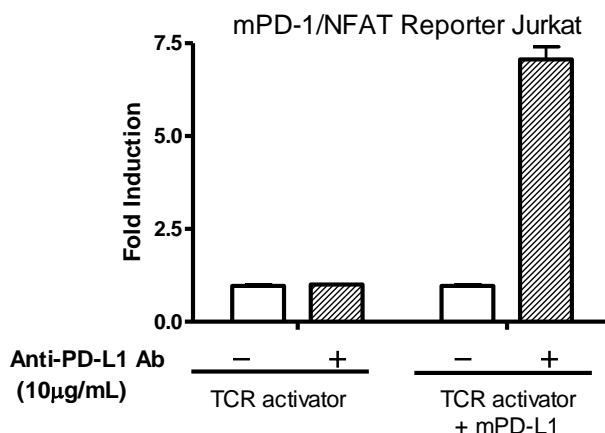
**C. Dose response of anti-mouse PD-1 neutralizing antibody in mouse PD-1/NFAT Reporter-Jurkat cells**



**Figure 2. Characterization of biological activity of anti-mouse PD-L1 neutralizing antibody in PD-1 /PD-L1 cell-based assay using the mouse PD-1/NFAT Reporter-Jurkat cells.**

HEK293 cells were transiently transfected with the vectors for mouse PD-L1 and the TCR activator. The next day, transfected HEK293 cells were pre-incubated with anti-mouse PD-L1 neutralizing antibody (Fisher Scientific #50-146-65, clone#MIH5) for 30 minutes prior to co-culture with mouse PD-1/NFAT Reporter-Jurkat cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to cells to measure NFAT activity.

**A. Anti-mouse PD-L1 neutralizing antibody induced NFAT luciferase reporter activity in mouse PD-1/NFAT Reporter-Jurkat cells co-cultured with HEK293 cells overexpressing mouse PD-L1 and TCR activator.**



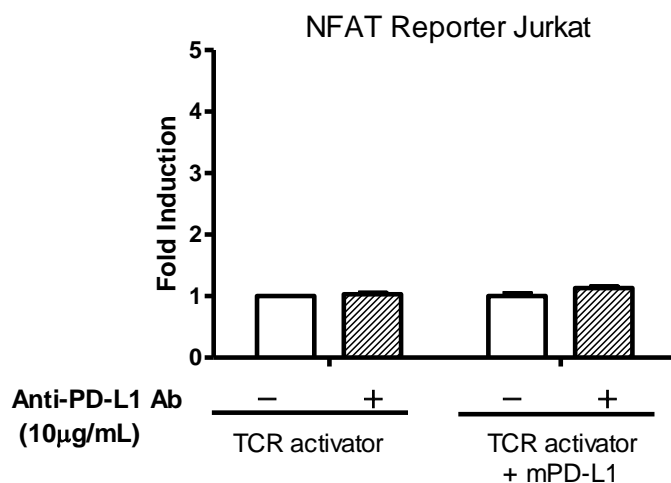
OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

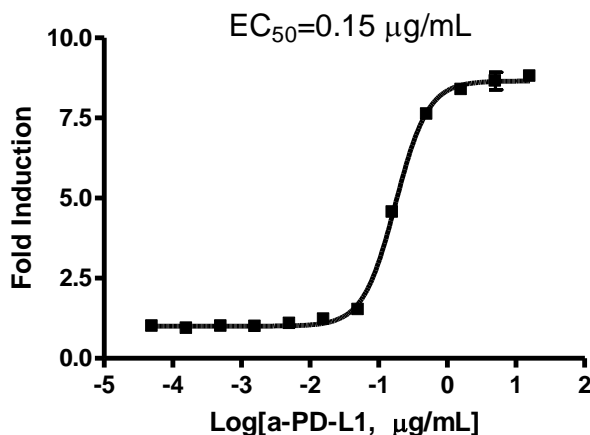
Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)

- B.** Anti-mouse PD-L1 neutralizing antibody had no effect on NFAT luciferase reporter activity in control NFAT Reporter-Jurkat cells (BPS Bioscience #60621) co-cultured with HEK293 cells overexpressing mouse PD-L1 and TCR activator.



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



OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.  
 To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**  
 Or you can Email us at: [info@bpsbioscience.com](mailto:info@bpsbioscience.com)  
 Please visit our website at: [www.bpsbioscience.com](http://www.bpsbioscience.com)



6042 Cornerstone Court W, Ste B  
San Diego, CA 92121  
**Tel:** 1.858.202.1401  
**Fax:** 1.858.481.8694  
**Email:** [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

## Related Products

| <b><u>Product</u></b>                                | <b><u>Cat. #</u></b> | <b><u>Size</u></b> |
|--|----------------------|--------------------|
| Mouse PD-1 / NFAT - Reporter - Jurkat Cell Line      | 79762                | 2 vials            |
| Mouse PD-L1/TCR-activator CHO cell line              | 79763                | 2 vials            |
| NFAT Reporter (Luc) – Jurkat Recombinant Cell Line   | 60621                | 2 vials            |
| ONE-Step™ Luciferase Assay System                    | 60690-1              | 10 ml              |
| ONE-Step™ Luciferase Assay System                    | 60690-2              | 100 ml             |
| Human PD-1 / NFAT - Reporter - Jurkat Cell Line      | 60535                | 2 vials            |
| Human PD-L1/TCR-activator CHO cell line              | 60536                | 2 vials            |
| Human PD-L1 / TCR Activator Mammalian Expression Kit | 60610                | 500 rxns           |
| Human PD-L2 / TCR Activator Mammalian Expression Kit | 60620                | 500 rxns           |

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: [\*\*info@bpsbioscience.com\*\*](mailto:info@bpsbioscience.com)

Please visit our website at: [\*\*www.bpsbioscience.com\*\*](http://www.bpsbioscience.com)