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Produktinformation

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

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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
TCR activator + Mouse PD-L1 (Component A)	Expression vectors constitutively expressing TCR activator and mouse 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

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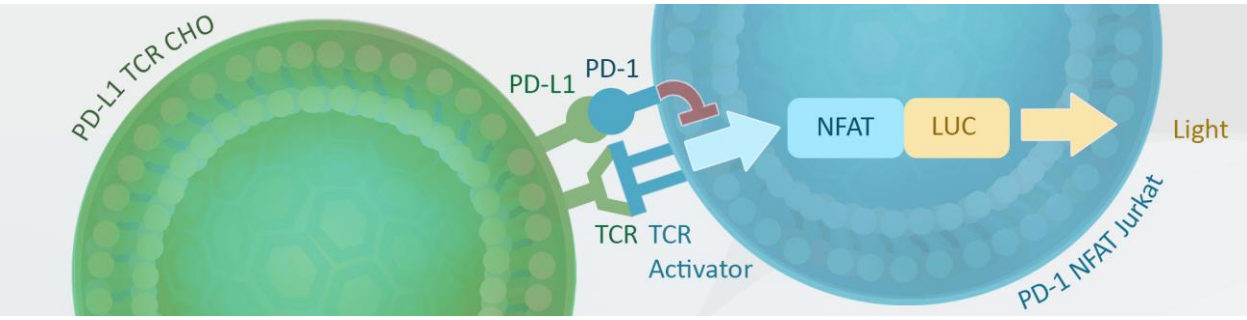
Functional Validation and Assay Performance

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

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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.

2. Next day, transfect 1 µl 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 x 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 µl 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 x10^4 /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 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.

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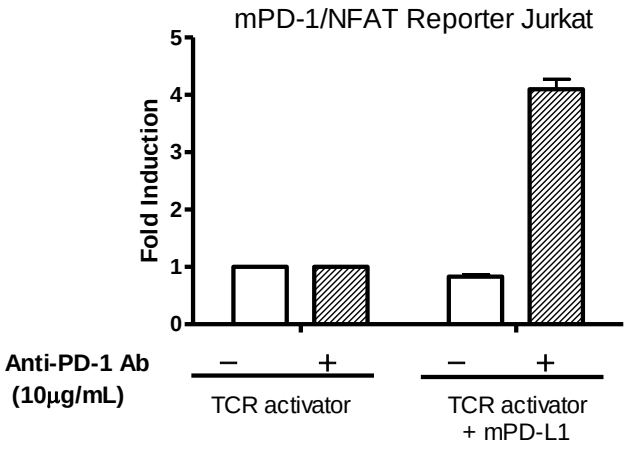
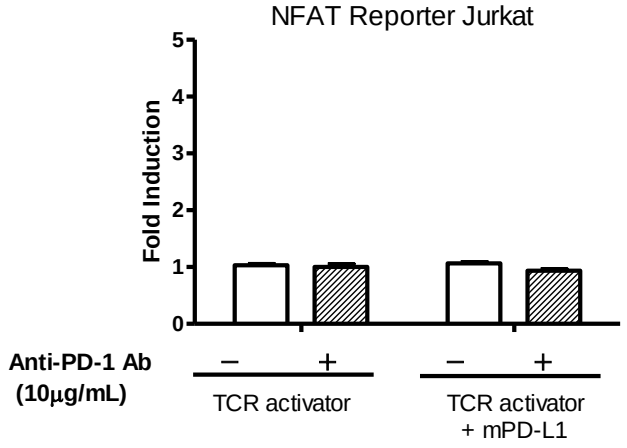
Figure 1. Characterization of biological activity of anti-mouse PD-1 neutralizing antibody in PD-1/PD-L1 cell-based assay using the mouse PD-1/NFAT Reporter-Jurkat cells.
HEK293 cells were transiently transfected with the vectors for mouse PD-L1 and the TCR activator. The next day, mouse PD-1/NFAT Reporter-Jurkat 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.



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

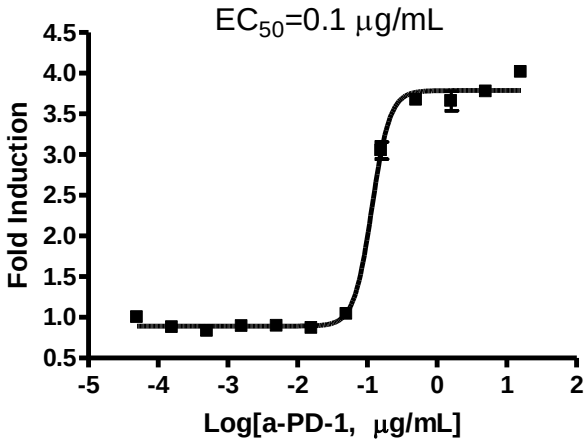
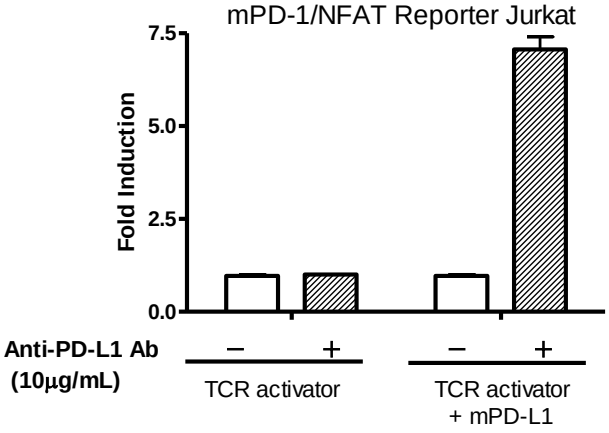


Figure 2. Characterization of biological activity of anti-mouse PD-L1 neutralizing antibody in PD-1 /PD-L1 cell-based assay using the mouse PD-1/NFAT Reporter-Jurkat cells.
HEK293 cells were transiently transfected with the vectors for mouse PD-L1 and the TCR activator. The next day, transfected HEK293 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.



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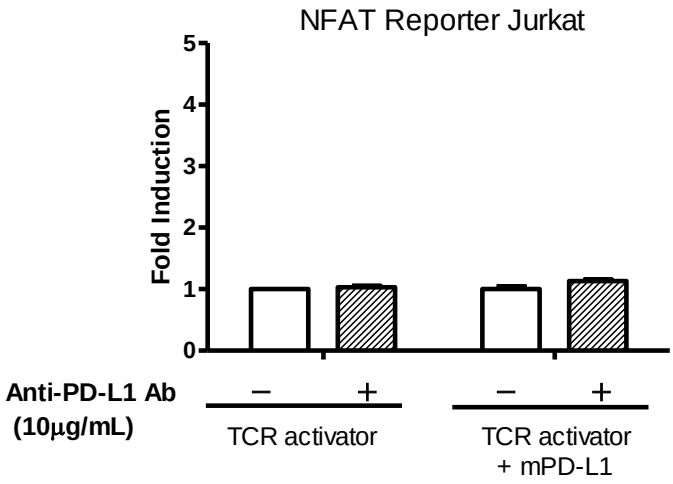
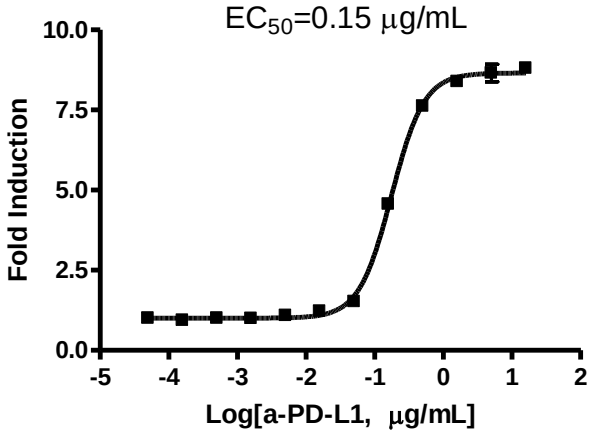
B. Anti-mouse PD-L1 neutralizing antibody had no effect on NFAT luciferase reporter activity in control NFAT Reporter-Jurkat cells (BPS Bioscience #60621) co-cultured with HEK293 cells overexpressing mouse PD-L1 and TCR activator.



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



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Related Products

Product	Cat. #	Size
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