

Data Sheet

TCR Activator / PD-L1 Mammalian Expression Kit Catalog #: 60610

Product Description

The recombinant expression vectors are designed to express human engineered T cell receptor (TCR) activator and human PD-L1 (GenBank Accession #NM_014143) in mammalian cells. The transfected cells can be used in conjunction with PD-1/NFAT Reporter/Jurkat T cells (cat #60535) 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 + Human PD-L1 (Component A)	Expression vectors constitutively expressing TCR activator and human 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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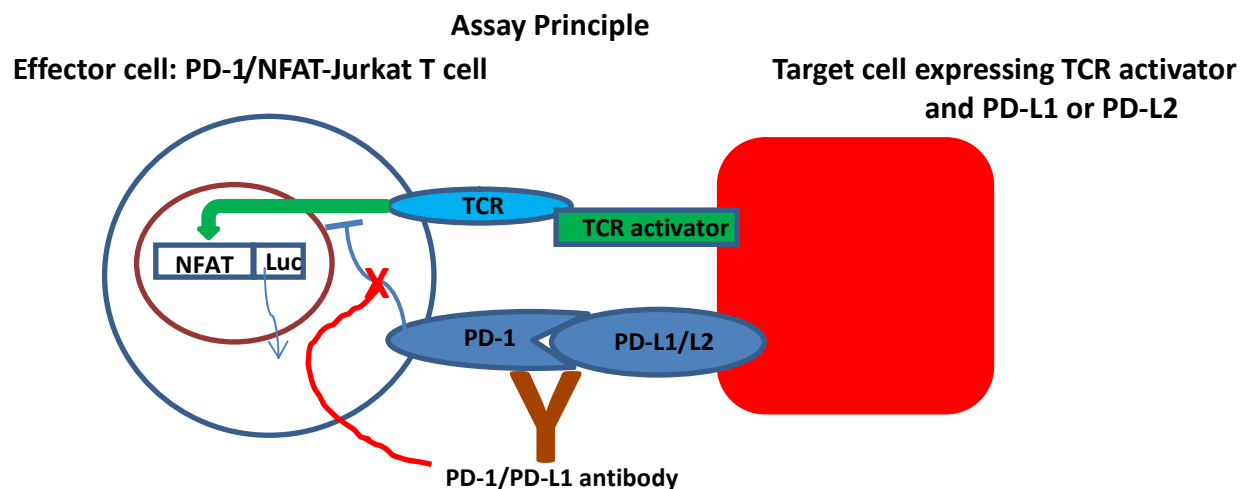
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Functional Validation and Assay Performance

In this assay, PD-1/NFAT Reporter/Jurkat T cells are used as effector cells; HEK293 cells over-expressing PD-L1 (or PD-L2) 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 (or PD-L2) 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.



Materials Required but Not Supplied

- HEK293 cell and its growth medium or other cell lines
- Transfection reagent for mammalian cell line
- PD-1/NFAT Reporter Jurkat T cells (cat #60535)
- Opti-MEM I Reduced Serum Medium (life technologies #31985-062)
- Assay medium: RPMI1640 + 10% FBS + 1% Penicillin/Streptomycin
- Anti-PD-1 neutralizing antibody: cat # 71120
- Anti-PD-L1 neutralizing antibody: cat #71213
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (# 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 μ l of growth medium so that cells will be 90% confluent at the time of transfection.
2. Next day, transfect 1 μ l of the expression vectors for TCR activator and human 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 PD-1/NFAT Reporter-Jurkat cells and the transfected HEK293 cells.

To test the anti-PD-1 antibody, dilute the antibody in assay medium, remove the medium from the PD-1/NFAT Reporter- Jurkat cells, and preincubate the anti-PD-1 antibody with transfected HEK293 cells for 30 minutes, then add the PD-1/NFAT Reporter- Jurkat cells to the transfected HEK293 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 transfected HEK293 for 30 min, then add the PD-1/NFAT Reporter- Jurkat to transfected HEK293.

4. After ~16 hours, measure the luciferase expression using the ONE-Step luciferase assay system: Add 100 μ l of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer. *If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.*
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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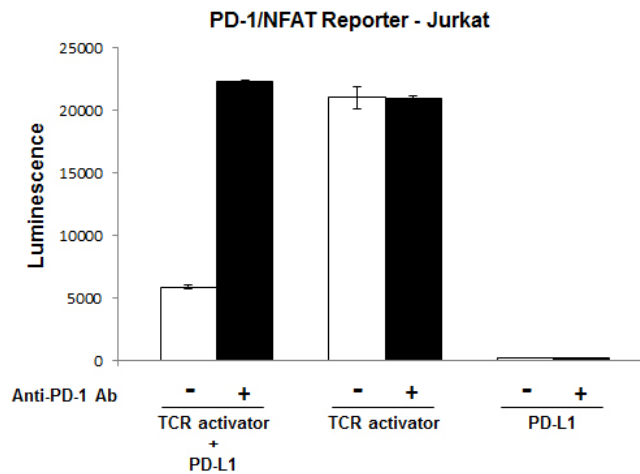


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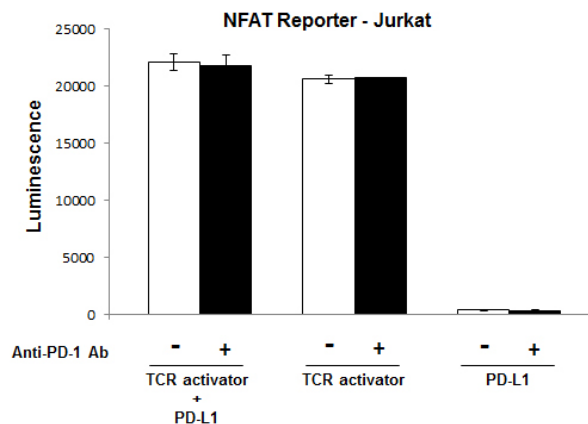
Figure 1. Characterization of biological activity of anti-PD-1 neutralizing antibody in PD-1/PD-L1 cell-based assay using the PD-1/NFAT Reporter-Jurkat cells.

HEK293 cells were transiently transfected with the vectors for human PD-L1 and the TCR activator. The next day, PD-1/NFAT Reporter-Jurkat cells (or control NFAT Reporter – Jurkat cells) were pre-incubated with anti-PD-1 neutralizing antibody (Cat # 71120) for 30 minutes prior to co-culture with transfected HEK293 cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent (Cat #60690) was added to the cells to measure NFAT activity.

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



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

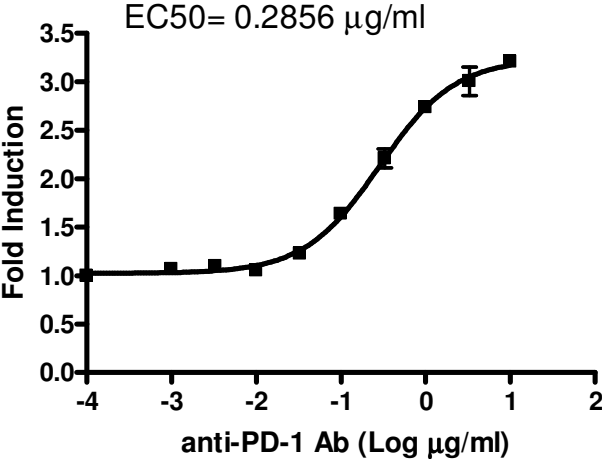
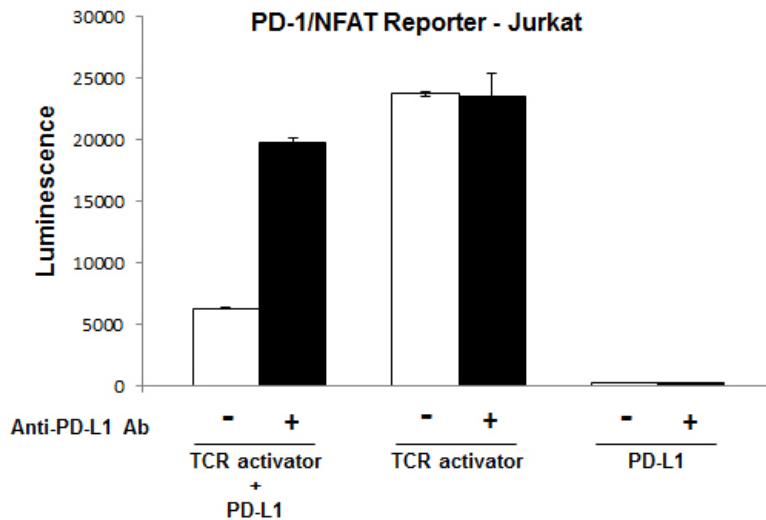


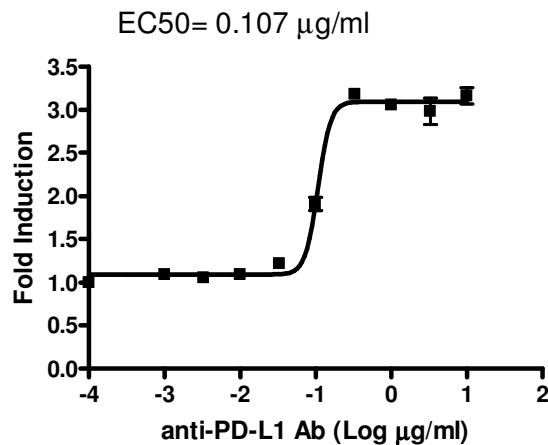
Figure 2. Characterization of biological activity of anti-PD-L1 neutralizing antibody in PD-1 /PD-L1 cell-based assay using the PD-1/NFAT Reporter-Jurkat cells.

HEK293 cells were transiently transfected with the vectors for human PD-L1 and the TCR activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-L1 neutralizing antibody (Cat # 71213) for 30 minutes prior to co-culture with PD-1/NFAT Reporter-Jurkat cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent (cat # #60690) was added to cells to measure NFAT activity.

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



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



Related Products

Product	Cat. #	Size
PD-1 / NFAT - Reporter - Jurkat Recombinant Cell Line	60535	2 vials
Anti-PD-1 neutralizing antibody	71120	100 µg
Anti-PD-L1 neutralizing antibody	71213	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

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