Data Sheet

TCR Activator / PD-L1 Mammalian Expression Kit
Catalog #: AMS.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 (#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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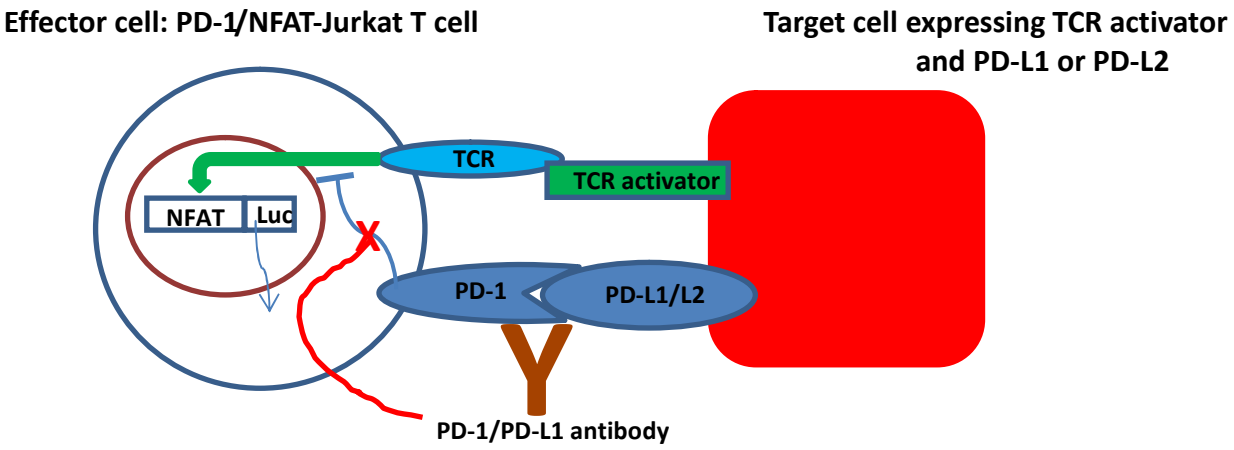
Functional Validation and Assay Performance

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.

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.]
- PD-1/NFAT Reporter Jurkat T cells (#60535)
- Opti-MEM I Reduced Serum Medium (life technologies #31985-062)
- Assay medium: RPMI1640 + 10% FBS + 1% Penicillin/Streptomycin
- Anti-PD-1 neutralizing antibody (#71120)
- Anti-PD-L1 neutralizing antibody (#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 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 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 µl 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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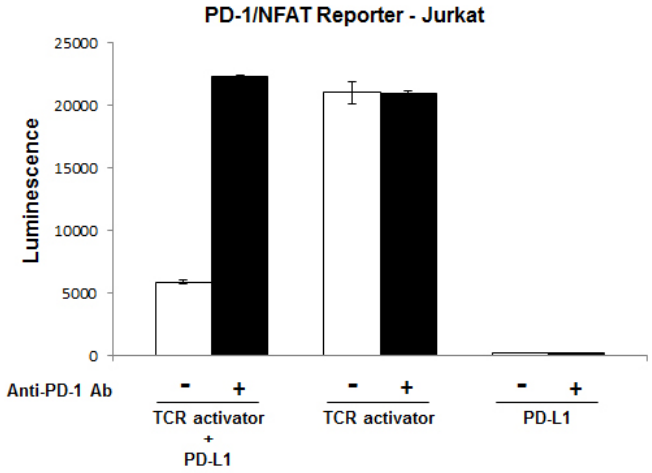
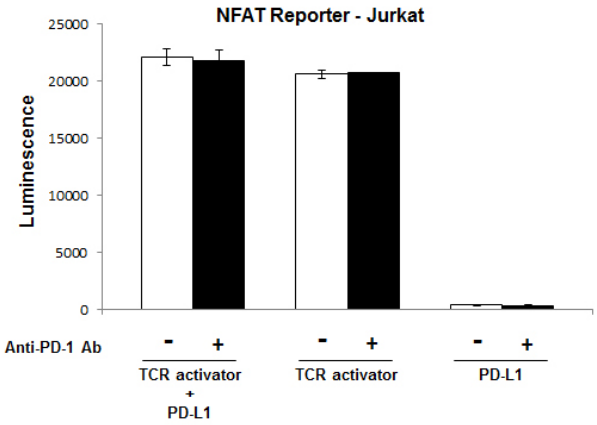
Figure 1. Characterization of biological activity of anti-PD-1 neutralizing antibody in PD-1/PD-L1 cell-based assay using the PD-1/NFAT Reporter-Jurkat cells.
HEK293 cells were transiently transfected with the vectors for human PD-L1 and the TCR activator. The next day, PD-1/NFAT Reporter-Jurkat cells (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.



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

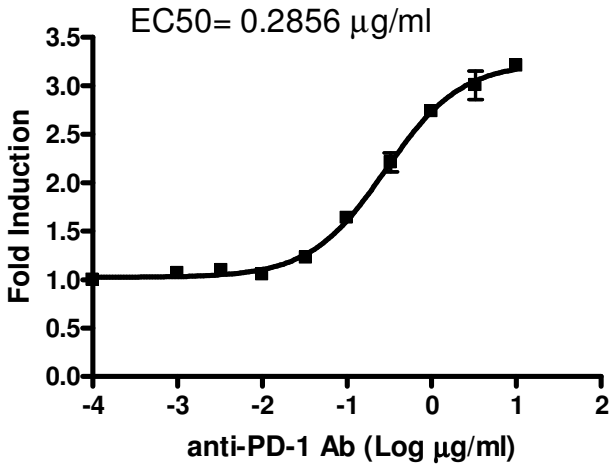

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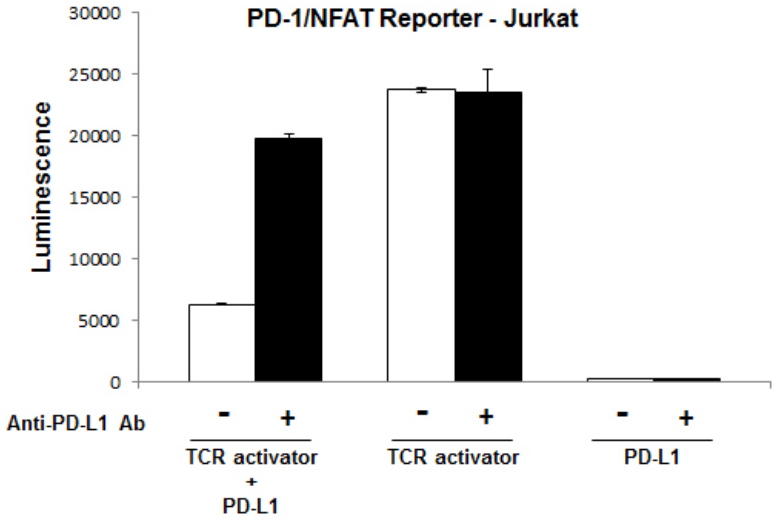
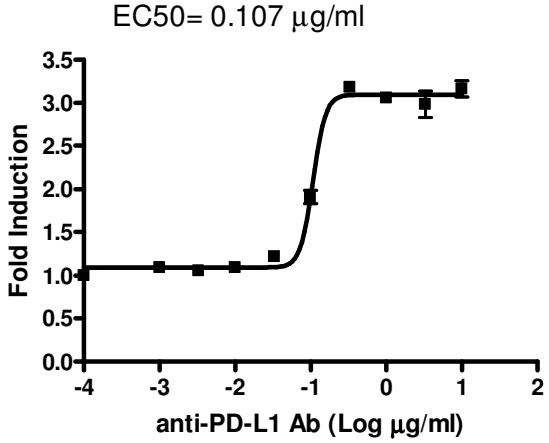
Figure 2. Characterization of biological activity of anti-PD-L1 neutralizing antibody in PD-1 /PD-L1 cell-based assay using the PD-1/NFAT Reporter-Jurkat cells.
HEK293 cells were transiently transfected with the vectors for human PD-L1 and the TCR activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-L1 neutralizing antibody (Cat. #71213) for 30 minu tes prior to co-culture with PD-1/NFAT Reporter-Jurkat cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent (BPS 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



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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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