Data Sheet

PD-1 (Mouse) / NFAT - Reporter - Jurkat Recombinant Cell Line
Catalog #: AMS.79762

Product Description
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of mouse PD-1 (Programmed Cell Death 1, PDCD1, SLEB2, CD279, GenBank Accession #NM_008798).

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 inhibitors of mouse PD-1 signaling in a cellular context
- Characterize the biological activity of mouse PD-1 and its interactions with ligands

Format
Each vial contains 2 x 10^6 cells in 1 ml of 90% FBS, 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions
Thaw Medium 2 (Cat. #AMS.60184): RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2F (Cat. #AMS.79669): Thaw Medium 2 (#AMS.60184), 1 mg/ml of Geneticin (Life Technologies #11811031), and 0.5 µg/ml of Puromycin (Takara, #631306).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2F.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **(no Geneticin and Puromycin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 **(no Geneticin and Puromycin)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. This cell line tends to grow more slowly than parental Jurkat cells. After 24 hours of culture, add an additional 3 – 4 ml of growth medium without antibiotics. At first passage, switch to Growth Medium 2F **(contains Geneticin and Puromycin)**. Cells should be split before they reach 2 x 10^6 cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

To freeze down the cells, count the cells, transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

Functional Validation and Assay Performance
Expression of mouse PD-1 in Jurkat cell line was confirmed by Flow Cytometry.

The functionality of the cell line was validated using a mouse PD-1: PD-L1 cell-based assay. In this assay, mouse PD-1/NFAT Reporter/Jurkat T cells are used as effector cells; CHO cells over-expressing murine PD-L1 and an engineered T cell receptor (TCR) activator are used as target cells (#AMS.79763). 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, PD-1 and PD-L1 ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by anti-mPD1 or anti-mPD-L1 antibodies. PD-1/PD-L1 neutralizing antibodies block PD-1:PD-L1 interaction and promote T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter.

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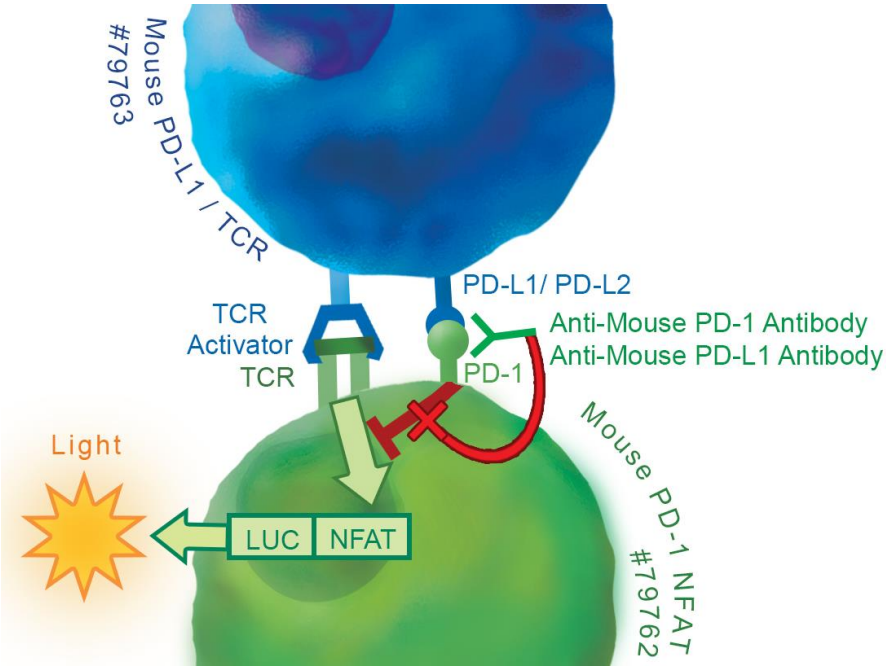
Assay Principle



Materials Required but Not Supplied

- Mouse PD-L1/TCR activator-CHO cell line (#AMS.79763)
- Assay medium: Thaw Medium 2 (#AMS.60184)
- Growth Medium 2F (#AMS.79669)
- Anti-mouse PD-1 neutralizing antibody: Bioxcell #BP0273, clone#29F.1A12
- Anti- PD-L1 neutralizing antibody (#AMS.71213)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (#AMS.60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer
- Thaw Medium 3 (#AMS.60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Protocol

1. Harvest Mouse PD-L1/TCR activator -CHO cells from culture and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 100 µl of Thaw Medium 3, #AMS.60186 (growth medium without Geneticin and HygromycinB). Incubate cells at 37°C in a CO_2 incubator for overnight. Cells should reach ~80% confluency on the next day (cells should not reach confluency in this step).

2. Next day, prepare serial dilution of anti-mouse PD-1 antibody or anti-mouse PD-L1 antibody in assay medium (Thaw Medium 2, #AMS.60184) (the concentrationof antibody here is 2x of the final treatment concentration of antibody). Harvest themouse PD-1/NFAT-reporter-Jurkat cells by centrifugation and resuspend in assaymedium. Dilute cells to 4 x 10^5 / ml in assay medium.

   To test anti-PD-1 antibody, 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 PD-L1/TCR activator -CHO cells and add 100 µl of PD-1/NFAT reporter – Jurkat cells / anti-PD-1 antibody mixture to the wells. (Note: *Mix the PD-1/NFAT Reporter- Jurkat cells with antibody well before adding to PD-L1/TCR activator-CHO cells.*)

   To test the anti-PD-L1 antibody, remove the medium from Mouse PD-L1/TCR activator -CHO cells, add 50 µl of diluted anti-PD-L1 antibody to the wells and incubate for 30 min. After incubation, add 50 µl of PD-1/NFAT Reporter- Jurkat cells (4 x 10^5 / ml) to the wells. (Note: *Mix the PD-1/NFAT Reporter- Jurkat cells well before adding to TCR activator/PD-L1-CHO cells.)*

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

   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

   Incubate the plates at 37°C in a CO_2 incubator for 5 to 6 hours.

3. After ~5 to 6 hours incubation, prepare ONE-Step™ luciferase assay reagents following recommended protocol. Add 100 µl of One-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.
   .
4. 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 treated well / average background-subtracted luminescence of untreated control wells.

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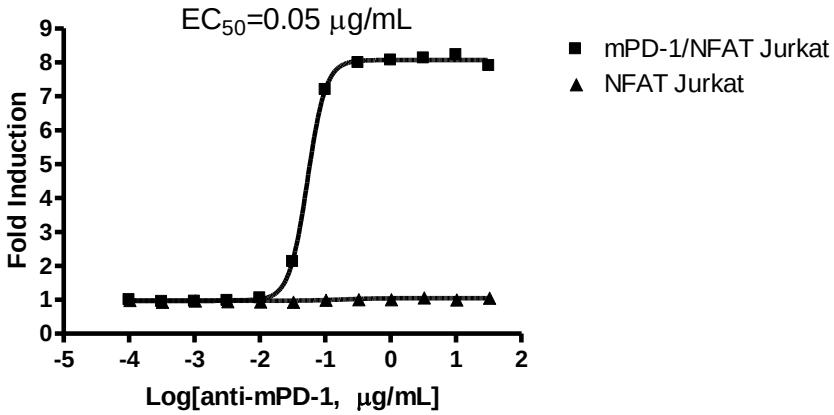

Figure 1. Cell Characterization Using a Mouse PD-1 Neutralizing Antibody. Mouse PD-L1/TCR activator-CHO cells were seeded in 96-well plate. The next day, Mouse PD-L1/TCR activator-CHO cells were incubated with anti-mouse PD-1 neutralizing antibody (Bioxcell #BP0273, clone#29F.1A12) and mouse PD-1/NFAT Reporter-Jurkat cells (or control NFAT Reporter – Jurkat cells, #AMS.60621). After incubation, ONE-Step™ Luciferase reagent (#AMS.60690) was added to the cells to measure NFAT activity. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells of each respective cell line.

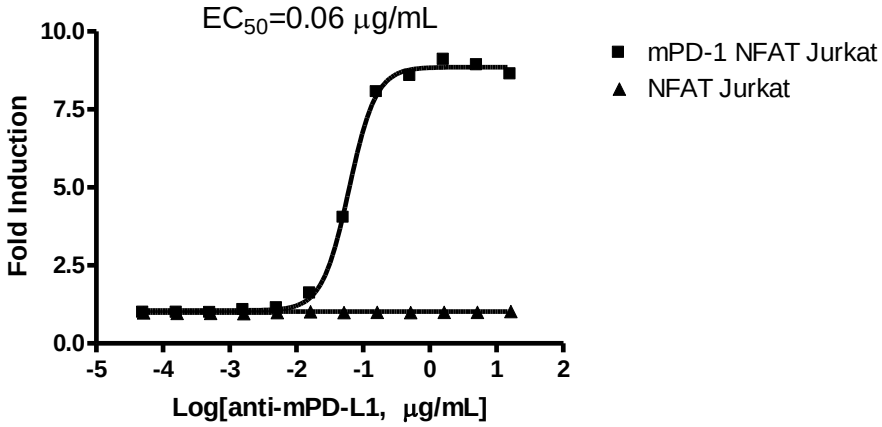

Figure 2. Cell Characterization Using a Mouse PD-L1 Neutralizing Antibody. Mouse PD-L1/TCR activator 1-CHO cells were seeded in 96-well plate. The next day, Mouse PD-L1/TCR activator -CHO cells were incubated with anti-PD-L1 neutralizing antibody and mouse PD-1/NFAT Reporter-Jurkat cells (or control NFAT Reporter – Jurkat cells, #AMS.60621). After incubation, ONE-Step™ Luciferase reagent (#AMS.60690) was added to cells to measure NFAT activity. The fold induction is equal to background-subtracted luminescence of antibody-treated well / background-subtracted luminescence of untreated-control wells of each respective cell line.

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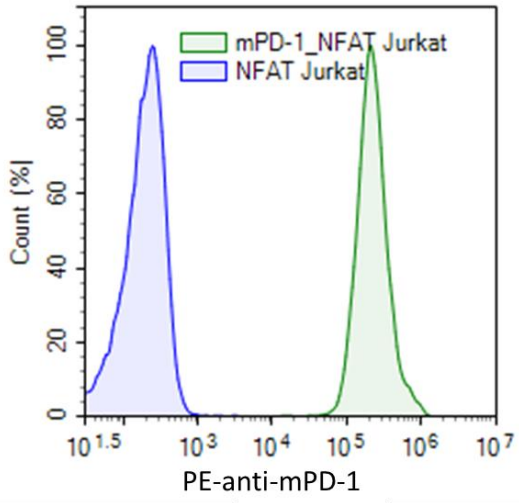

Figure 3. FACS analysis of cell surface expression of mouse PD-1 in mouse PD-1/NFAT Reporter-Jurkat cells. Mouse PD-1/NFAT Reporter-Jurkat cells or control NFAT Reporter-Jurkat cells were stained with PE-labeled anti-mouse PD-1 antibody (Fisher Scientific #50-113-42, clone#J43) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.

Sequence

mPD-1 sequence (accession number NM_008798)

MWVRQVPWSFTWAVLQLSWQSGWLLEVPNGPWRSLTFYPAWLTVSEGANATFTCSLSNWSEDLM
LNWNRLSPSNQTEKQAAFCNGLSQPVQDARFQIIQLPNRHDFHMNILDTRRNDSGIYLCGAISL
HPKAKIEESPGAELVVTERILETSTRYPSPSPKPEGRFQGMVIGIMSALVGIPVLLLLAWALAV
FCSTSMSEARGAGSKDDTLKEEPSAAPVPSVAYEELDFQGREKTPELPTACVHTEYATIVFTEG
LGASAMGRRGSADGLQGPRPPRHEDGHCSWPL

Related Products

Product	Cat. #	Size
NFAT Reporter – Jurkat cell line	60621	2 vials
Mouse PD-L1/TCR activator-CHOcell line	79763	2 vials
PD-1/NFAT Reporter-Jurkat cell line	60535	2 vials
PD-L1/TCR-activator CHO cell line	60536	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Mouse PD-1 (CD279), Fc fusion	71116	100 µg
Mouse PD-1, Avi-His-tag	79288	100 µg
Mouse PD-1, Avi-His-tag, Biotin-labeled	79189	50 µg
Mouse PD-1[Biotinylated]:PD-L1 Inhibitor Assay Kit	72027	96 rxns

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