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Produktinformation

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

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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

TCR activator / PD-L1 - CHO Recombinant Cell line
Cat. #: 60536

PRODUCT DESCRIPTION:
Recombinant CHO-K1 cells constitutively expressing human PD-L1 (Programmed Cell Death 1 Ligand 1, CD274**,** B7 homolog 1 (B7- H1), GenBank accession # NM_014143) and an engineered T cell receptor (TCR) activator.

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.

APPLICATIONS:

- Screen for activators or inhibitors of PD-1 signaling in a cellular context
- Screen PD-L1 antibodies for binding affinity
- Characterize the biological activity of PD-1 interactions with PD-L1

FORMAT:
Each vial contains 2.5 x 10^6 cells in 1 ml of 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) to confirm the absence of *Mycoplasma* species.

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GENERAL CULTURE CONDITIONS:

Thaw Medium 3 (BPS Bioscience #60186): Ham's F-12 medium (Hyclone # SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 3A (BPS Bioscience #60188): Thaw Medium 3 (BPS Bioscience #60186) plus 1 mg/ml of Geneticin (Life Technologies #11811031) and 500 µg/ml of Hygromycin B (Hyclone #SV30070.01) to ensure recombinant expression. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3A. TCR activator / PD-L1 – CHO cells should exhibit a typical cell division time of ~24 hours.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 3 **(no Geneticin and Hygromycin B)**, spin down cells, re-suspend cells in pre-warmed Thaw Medium 3 **(no Geneticin and Hygromycin B)**, transfer re-suspended cells to T25 flask and culture in 37°C CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 **(no Geneticin and Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch to Growth Medium 3A **(contains Geneticin and Hygromycin B)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 3A and transfer to a tube, spin down cells, re-suspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:10 to 1:20 twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 3 **(no Geneticin or Hygromycin B)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2x10(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 human PD-L1 in CHO-K1 cells was confirmed by Western blotting and FACS. The functionality of the cell line was validated using a PD-1:PD-L1 cell-based assay. In this assay, Jurkat T cells expressing NFAT reporter with constitutive expression of PD-1 (PD-1/NFAT Reporter/Jurkat, BPS# 60535) are used as effector cells; TCR activator / PD-L1- CHO cells 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. PD1/PD-L1 neutralizing antibodies block PD1:PD-L1 interaction and promote T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter.

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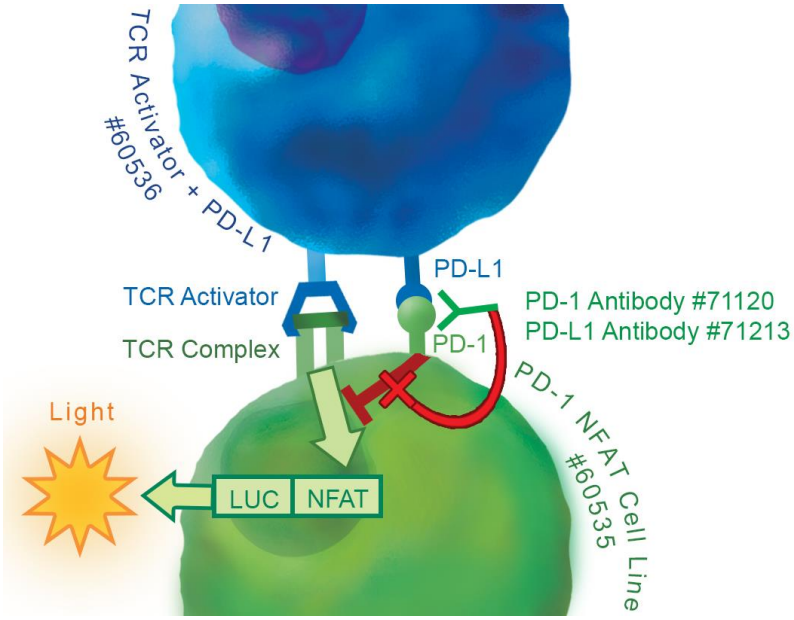
ASSAY PRINCIPLE



MATERIALS REQUIRED BUT NOT SUPPLIED:

- PD-1/NFAT reporter-Jurkat cell line (BPS Bioscience # 60535)
- Assay medium: Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 3A (BPS Bioscience #60188)
- Anti-PD-1 neutralizing antibody: BPS Bioscience #71120
- Anti-PD-L1 neutralizing antibody: BPS Bioscience #71213
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience # 60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer
- Thaw Medium 3 (BPS Bioscience #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 TCR activator / PD-L1-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, BPS Bioscience #60186 (growth medium without Geneticin and Hygromycin B). Incubate cells at 37° in a CO2 incubator for overnight. Cells should reach ~80% confluency on the next day (cells should not reach confluency in this step).

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2. Next day, prepare serial dilution of anti-PD-1 antibody or anti-PD-L1 antibody in Thaw Medium 2, BPS Bioscience #60184 (assay medium) (the concentration of antibody here is 2x of the final treatment concentration of antibody). Harvest the PD-1/NFAT-reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute cells to $4x10^5$ / ml in assay medium.

   To test anti-PD-1 antibody, preincubate the PD-1/NFAT Reporter- Jurkat cells ($4x10^5$ / ml) with diluted anti-PD-1 antibody (1:1 in volume) for 30 min. After incubation, remove the medium from TCR activator/PD-L1-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 TCR activator/PD-L1-CHO cells.*)

   To test the anti-PD-L1 antibody, remove the medium from TCR activator/PD-L1-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 ($4x10^5$ / 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 PD-1/NFAT Reporter- Jurkat cells is 2 $x10^4$ /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° in a CO2 incubator for 5 to 6 hours.

3. After ~5 to 6-hour incubation, perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of One-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

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.

Figure 1. Cell Characterization Using a PD-1 Neutralizing Antibody. TCR activator/PD-L1-CHO cells were seeded in 96-well plate. The next day, TCR activator/PD-L1-CHO cells were incubated with anti-PD-1 neutralizing antibody (BPS Bioscience #71120) and PD-1/NFAT Reporter-Jurkat cells (BPS Bioscience #60535) (or control NFAT Reporter – Jurkat cells, BPS Bioscience #60621). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure NFAT activity.

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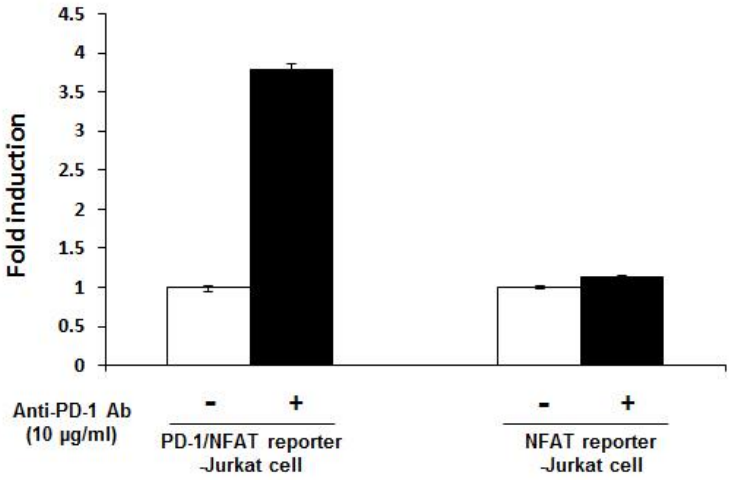
The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells of each respective cell line.

A. Anti-PD-1 neutralizing antibody induced NFAT luciferase reporter activity in PD-1/NFAT Reporter-Jurkat cells, but not NFAT Reporter – Jurkat cells, co-cultured with TCR activator/PD-L1-CHO cells.



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

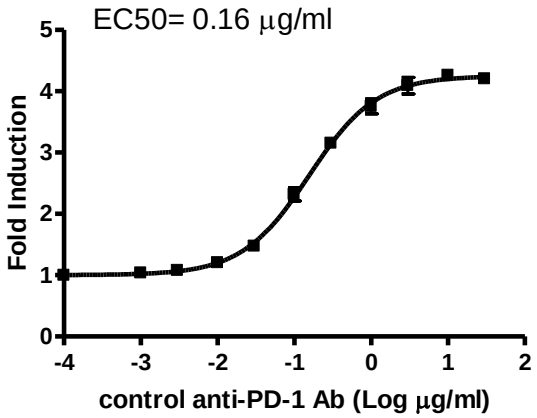


Figure 2. Cell Characterization Using a PD-L1 Neutralizing Antibody TCR activator/PD-L1-CHO cells were seeded in 96-well plate. The next day, TCR activator/PD-L1-CHO cells were incubated with anti-PD-L1 neutralizing antibody (BPS Bioscience #71213) and PD-1/NFAT Reporter-Jurkat cells (BPS Bioscience # 60535) (or control NFAT Reporter – Jurkat cells, BPS Bioscience #60621). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to cells to measure NFAT activity.

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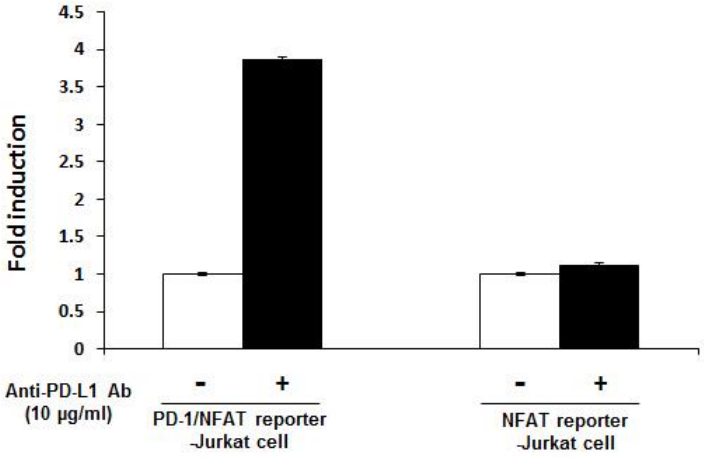
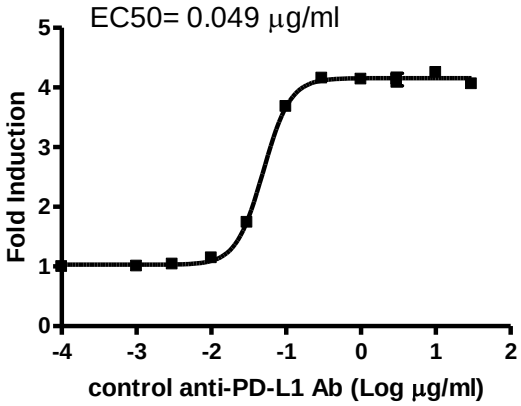
The fold induction is equal to background-subtracted luminescence of antibody-treated well / background-subtracted luminescence of untreated-control wells of each respective cell line.

A. Anti-PD-L1 neutralizing antibody induced NFAT luciferase reporter activity in PD-1/NFAT Reporter-Jurkat cells, but not NFAT Reporter – Jurkat cells, co-cultured with TCR activator / PD-L1 – CHO cells.



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



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SEQUENCE:

hPD-L1 sequence (accession number NM_014143)

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNI
IQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITV
KVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFN
VTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALT
FIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

RELATED PRODUCTS:

Product	**Cat. #**
PD-1/NFAT Reporter-Jurkat cell line	60535
NFAT Reporter – Jurkat cell line	60621
TCR activator-CHO cell line	60539
Anti-PD-1 neutralizing antibody	71120
Anti-PD-L1 neutralizing antibody	71213
TCR activator / PD-L1 expression kit	60610
TCR activator / PD-L2 expression kit	60620
ONE-Step™ Luciferase Assay System	60690-1
ONE-Step™ Luciferase Assay System	60690-2
Human PD-1 (CD279), Fc fusion	71106
Human PD-1, FLAG-Avi-His-tag	71198
Human PD-L1 (CD274), Fc fusion	71104
Human PD-L1 (CD274), FLAG-Avi-His tag	71183
Human PD-L2 (CD273), Fc fusion	71107
Human PD-1, Fc fusion, Biotin-labeled	71109
Human PD-L1, Fc fusion, Biotin-labeled	71105
Anti-PD-L1 Antibody, PE-labeled	71128