Description

The PD-1:PD-L1/PD-L2 Cell-Based Inhibitor Screening Assay is a bioluminescent cell-based assay for screening and profiling inhibitors of the PD-1:PD-L1 or PD-1:PD-L2 interaction. The assay consists of two main components: effector cells and expression vectors encoding TCR activator, human PD-L1, and human PD-L2. Growth-Arrested PD-1 Effector Cells (PD-1/NFAT Reporter Jurkat cells) are Jurkat cells expressing firefly luciferase, under the control of NFAT response elements, and human PD-1. These cryopreserved cells are provided in a thaw-and-use format that does not require cell propagation. These cells cannot be expanded and are intended to be single use. The expression vectors for TCR Activator, human PD-L1, and human PD-L2 are transfection-ready vectors that can be used to transfect cells and create target cells that overexpress PD-L1 or PD-L2 and an engineered cell surface T cell receptor (TCR) activator. The kit contains enough cells and plasmids for 100 assays if using a 96-well plate.

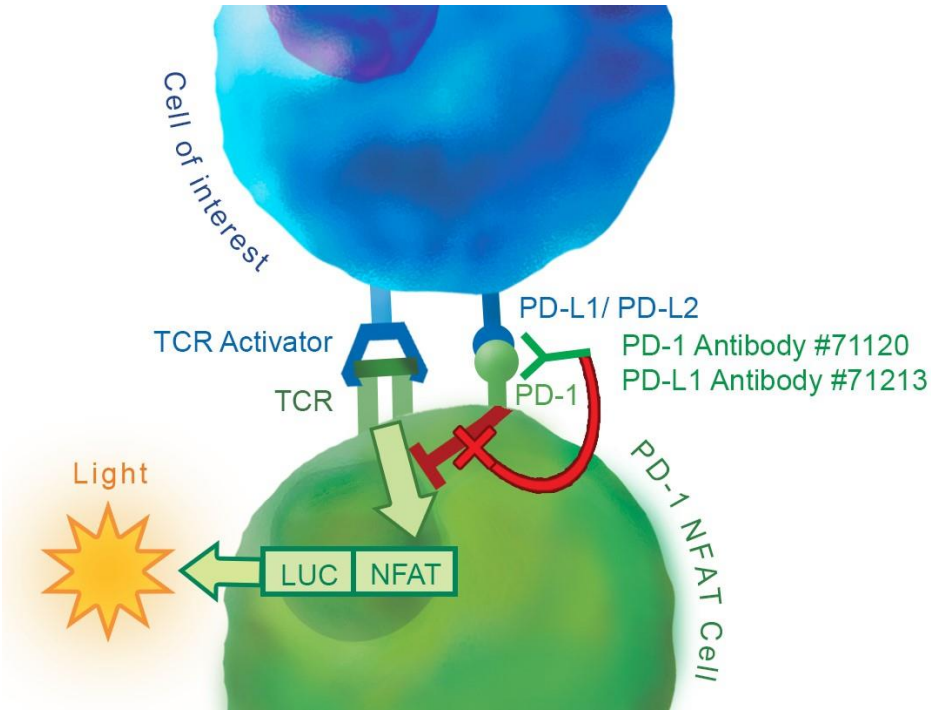

Figure 1: Illustration of the principle of *the PD-1:PD-L1/PD-L2 Cell-Based Inhibitor Screening Assay*. Jurkat T cells expressing NFAT reporter and PD-1 (PD-1/NFAT Reporter Jurkat Cells) act as effector cells. When co-cultivated, TCR complexes on PD-1/NFAT Reporter Jurkat cells are activated by the TCR activator expressed in the transfected cell line of interest, resulting in expression of the NFAT luciferase reporter. However, PD-1 and PD-L1/ PD-L2 ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by anti- PD-1 or anti- PD-L1/PD-L2 antibodies. Neutralizing antibodies block PD-1:PD-L1/PD-L2 interaction and result in reactivation of the NFAT responsive luciferase reporter.

Background

PD-L1 and PD-L2 binding to PD-1, a receptor expressed on T-cells, negatively regulates immune responses. PD-1 ligands PD-L1 and PD-L2 are found on the surface of many cancer cells, and their interaction with receptor PD-1 inhibits T cell activity and allows cancer cells to escape immune surveillance. This pathway is also involved in regulating autoimmune responses. Therefore, these proteins (termed immune checkpoints) are promising therapeutic targets for many types of cancer as well as multiple sclerosis, arthritis, lupus, and type I diabetes. Checkpoint inhibitors have remarkable efficacy in a wide range of cancer types and have revolutionized cancer treatment. PD-1 inhibitors nivolumab, pembrolizumab, cemiplimab and PD-L1 inhibitors atezolizumab, avelumab, and durvalumab are all FDA-approved drugs for immuno-therapy.

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Application

- Screen for compound activity on of PD-1 or PD-L1/PD-L2 signaling in a cellular model.
- Characterize the biological activity of PD-1 and its interactions with ligands.

Supplied Materials

Catalog #	Name	Amount	Storage
	Growth-Arrested PD-1 Effector Cells (PD-1/NFAT reporter-Jurkat cell)	1 Vial	LN_2
60610	TCR Activator + Human PD-L1 (expression vectors for expressing TCR Activator and human PD-L1)	100 µl	-20°C
60620	TCR Activator + Human PD-L2 (expression vectors for expressing TCR Activator and human PD-L2)	100 µl	-20°C
60184	Thaw Medium 2	50 ml	4°C
60690	ONE-Step™ Luciferase Assay	10 ml	-20°C
71120	Anti-PD-1 Neutralizing Antibody	10 µl	-80°C

Materials Required but Not Supplied

- Cell line of interest, such as HEK293 cells, and corresponding culture media
- Transfection Reagent, such as Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher #11668027)
- Opti-MEM I Reduced Serum Medium (Thermo Fisher #31985-062)
- 96-well tissue culture-treated white clear-bottom assay plate
- Luminometer

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Assay Protocol

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The protocol described is designed for HEK293 as cells of interest. The use of other cells may require optimization.
- The transfection protocol is a general guideline using Lipofectamine™ 2000 and we recommend referring to the manufacturer's instructions for updated and detailed information.
- The experiments should be performed in triplicate.
- The assay should include "Cell-Free Control", "Unstimulated Control" and "Test Condition" wells.

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A. Effect of anti-PD-1 Antibody on PD-1:PD-L1/ PD-L2 binding.

Day 1:

1. Seed HEK293 cells, at a density of ~30,000 cells in 100 µl/well of appropriate cell culture media into a white clear-bottom 96-well cell culture plate. Leave some wells with only assay medium for background determination.

   Note: This cell density should result in HEK293 being 90% confluent the next day.

2. Incubate cells at 37°C with 5% CO_2 overnight.

Day 2:

3. Transfect each well with 1 µl of TCR Activator + Human PD-L1 or TCR Activator + Human PD-L2, following the transfection reagent's manufacturer's protocol. The protocol described below is a guideline only for use of Lipofectamine™ 2000 and may require optimization to obtain the appropriate transfection efficiency and low cell toxicity.

   a. Mix 1 µl of DNA with 15 µl of antibiotic-free Opti-MEM I Reduced Serum Medium.

   b. Prepare a mix of 0.3 µl of Lipofectamine ™ 2000 with 15 µl of Opti-MEM I Reduced Serum Medium for each reaction.

   c. Incubate for 5 minutes at Room Temperature (RT).

   d. Combine the diluted DNA with diluted Lipofectamine™ 2000 (15 µl + 15 µl).

   e. Mix gently and incubate 25 minutes at RT.

   f. Add DNA/Lipofectamine™ 2000 mix to cells (30 µl/ well).

   g. Incubate cells at 37°C in a CO2 incubator for 6-24 hours.

   h. Gently remove spent media and replenish with fresh media.

   i. Visualize cells under a microscope for signs of cell toxicity. If needed, allow cells to recover for an extra 24 hours.

Day 3:

4. Quickly thaw the Growth-Arrested PD-1 Effector cells in a 37°C water-bath and transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2.

5. Spin down the cells at 1500 rpm, remove supernatant and re-suspend cells in 7 ml of pre-warmed Thaw Medium 2.

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6. Prepare a serial dilution of the anti-PD-1 antibody at 2x the desired final concentration in Thaw Medium 2 (25 µl/well).

7. Pre-incubate Growth-Arrested PD-1 Effector cells with the diluted anti-PD-1 antibody (1:1 volume ratio) for 15-30 minutes (50 µl of mix/well).

8. Remove media carefully from the transfected HEK293-containing wells.

9. Add 50 µl of fresh assay medium to the wells containing the transfected HEK293 cells.

10. Add the mixture of Growth-Arrested PD-1 Effector cells/antibody to the transfected HEK293 cells (50 µl/well).

11. Add 100 µl of assay medium to the "Unstimulated Control" and "Cell-Free Control" wells (for determining the background luminescence).

12. Incubate the cells at 37°C in a CO_2 incubator for 16 hours.

13. Add 100 µl of ONE-Step™ Luciferase Assay reagent to all wells.

14. Rock at room temperature for ~15 minutes.

15. Measure luminescence using a luminometer.

16. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of luciferase activity is the background-subtracted luminescence of stimulated cells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Lumin.\ stimulated\ cells - ave.\ background}{Lumin.\ unstimulated\ cells - ave.\ background}$$

B. Effect of anti-PD-L1/PD-L2 Antibody on PD-1:PD-L1/ PD-L2 binding.

Day 1:

1. Seed HEK293 cells, at a density of ~30,000 cells in 100 µl/well of appropriate cell culture media into a white clear-bottom 96-well cell culture plate.
   Note: This cell density should result in HEK293 being 90% confluent the next day.

2. Incubate cells at 37°C with 5% CO_2 overnight.

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Day 2:

3. Transfect cells with 1 µl of TCR Activator + Human PD-L1 or TCR Activator + Human PD-L2, following the transfection reagent's manufacturer's protocol. The protocol described below is a guideline only for use of Lipofectamine™ 2000 and may require optimization to obtain the appropriate transfection efficiency and low cell toxicity.

    a. Mix 1 µl of DNA with 15 µl of antibiotic-free Opti-MEM I Reduced Serum Medium.

    b. Prepare a mix of 0.3 µl of Lipofectamine ™ 2000 with 15 µl of Opti-MEM I Reduced Serum Medium for each reaction.

    c. Incubate for 5 minutes at Room Temperature (RT).

    d. Combine the diluted DNA with diluted Lipofectamine™ 2000 (15 µl + 15 µl).

    e. Mix gently and incubate 25 minutes at RT.

    f. Add DNA/Lipofectamine™ 2000 mix to cells (30 µl/ well).

    g. Incubate cells at 37°C in a CO_2 incubator for 6-24 hours.

    h. Gently remove spent media and replenish with fresh media.

    i. Visualize cells under a microscope for signs of cell toxicity. If needed, allow cells to recover for an extra 24 hours.

Day 3:

4. Quickly thaw the Growth-Arrested PD-1 Effector cells in a 37°C water-bath and transfer the entire contents of the vial to a tube containing 10 ml of assay medium.

5. Spin down the cells at 1500 rpm, remove supernatant and re-suspend cells in 7 ml of pre-warmed Thaw Medium 2.

6. Prepare a serial dilution of the anti-PD-L1/PD-L2 antibody at 2x the desired final concentration in Thaw Medium 2 (50 µl/well).

7. Remove media carefully from the transfected HEK293-containing wells.

8. Add 50 µl of diluted antibody to the transfected HEK293 containing wells.

9. Incubate for 15-30 minutes.

10. Add 50 µl of Growth-Arrested PD-1 Effector cells to the wells with HEK293 and antibody.

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11. Add 100 µl of assay medium to the "Unstimulated Control" and "Cell-Free Control" wells (for determining the background luminescence).

12. Incubate the cells at 37°C in a CO_2 incubator for 16 hours.

13. Add 100 µl of ONE-Step™ Luciferase Assay reagent to all wells.

14. Rock at room temperature for ~15 minutes.

15. Measure luminescence using a luminometer.

17. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of luciferase activity is the background-subtracted luminescence of stimulated cells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Lumin.\ stimulated\ cells - ave.\ background}{Lumin.\ unstimulated\ cells - ave.\ background}$$

Validation Data

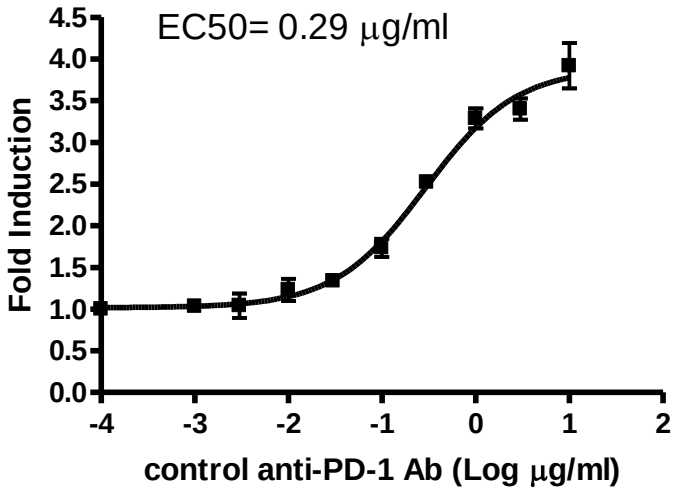

Figure 2. Dose response curve of HEK293 cells transfected with TCR Activator and human PD-L1 to anti-PD-1 antibody using the PD-1:PD-L1/PD-L2 Cell-Based Inhibitor Screening Assay.
HEK293 cells were transiently transfected with human PD-L1 and TCR Activator. The next day, growth-arrested PD-1 Effector cells were pre-incubated with the Anti-PD-1 Neutralizing Antibody (BPS Bioscience #71120) for 30 minutes prior to co-culture with transfected HEK293 cells. After ~16 hours NFAT activity was measured using ONE-Step™ Luciferase. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells.

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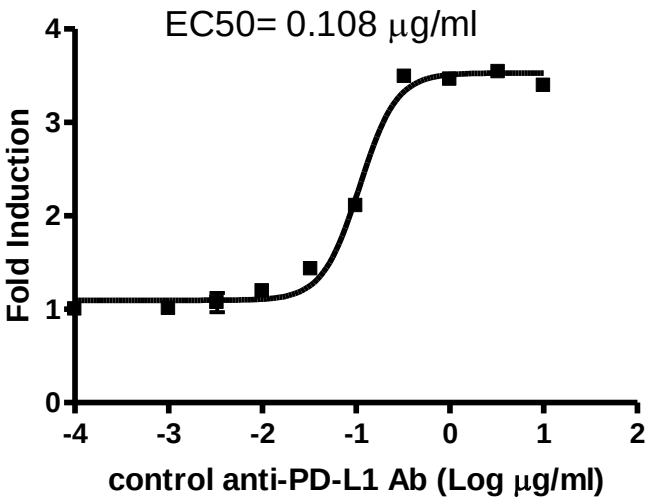

Figure 3. Dose response curve of HEK293 cells transfected with TCR Activator and human PD-L1 to anti-PD-L1 antibody using the PD-1:PD-L1/PD-L2 Cell-Based Inhibitor Screening Assay.
HEK293 cells were transiently transfected with human PD-L1 and TCR Activator. The next day, transfected HEK293 cells were pre-incubated with Anti-PD-L1 Neutralizing antibody (BPS Bioscience #71213) for 30 minutes prior to co-culture with Growth-Arrested PD-1 Effector cells. After ~16 hours, NFAT activity was measured using ONE-Step™ Luciferase. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Sasca D, et al. (2019) Blood 133: 2305-2319.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
PD-1/NFAT Reporter Jurkat Cell Line	60535	2 vials
NFAT Luciferase Reporter Jurkat Cell Line	60621	2 vials
PD-L1 /TCR Activator CHO Cell Line	60536	2 vials
TCR Activator CHO Cell Line	60539	2 vials
Anti-PD-1 Neutralizing Antibody	71120	100 µg
Anti-PD-L1 (CD274) Neutralizing Antibody	71213	100 µg

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