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Produktinformation

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

Weitere Information auf den folgenden Seiten!
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

PD-1:PD-L1/PD-L2 Cell-Based Inhibitor Screening Assay Kit
Catalog #: 60800

Background
Programmed Cell Death Protein 1 (PD-1) is a receptor which is expressed on activated T-cells. Binding of PD-1 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.

Description
The PD-1/PD-L1 Inhibitor Screening Cell-Based Assay is a bioluminescent cell-based assay that can be used to screen and profile inhibitors of PD-1:PD-L1 or PD-1:PD-L2 interaction. The assay consists of two main components:

• Growth-Arrested PD-1 Effector cells (PD-1/NFAT reporter-Jurkat cells): Reporter Jurkat T cells expressing firefly luciferase gene under the control of NFAT response elements and also constitutively expressing human PD-1. These cryopreserved cells are provided in a thaw-and-use format that does not require cell propagation. These cells are not to be expanded and are intended to be used in a single experiment.

• Expression vectors for TCR activator, human PD-L1, and human PD-L2: Transfection-ready vectors are used to transfect cells to create the target cells that overexpress PD-L1 or PD-L2 and an engineered cell surface T cell receptor (TCR) activator.

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

Storage
Store cell line in liquid nitrogen immediately upon receipt. Store other components as described below. Stable for at least 6 months from date of receipt when stored as directed.

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.]
- Opti-MEM I Reduced Serum Medium (Life technologies #31985-062)
- 96-well tissue culture-treated white clear-bottom assay plate
- Luminometer

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Components

Cat. #	Component	Specification	Amount	Storage
	Growth-Arrested PD-1 Effector Cells	PD-1/NFAT reporter-Jurkat cell	1 vial	Liquid Nitrogen
60610	**TCR activator + Human PD-L1**	Expression vectors constitutively expressing TCR activator and human PD-L1	100 µl	-20°C
60620	**TCR activator + Human PD-L2**	Expression vectors constitutively expressing TCR activator and human PD-L2	100 µl	-20°C
60184	**Thaw Medium 2**	Assay medium specially formulated for use in this assay.	50 ml	4°C
60690	**ONE-Step™ Luciferase Assay**	Luciferase Reagent Buffer (component A) + Luciferase Reagent Substrate(component B)	10 ml	-20°C
71120	**Anti-PD-1 neutralizing antibody**	PD-1-binding neutralizing antibody for use as control.	10 µg	-80°C

Each kit contains sufficient reagents for 100 assays using a 96-well plate.

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 by transient transfection are used as target cells. When the 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. In both scenarios, this inhibition can be specifically reversed by anti-PD1 antibodies. Depending on which construct is used, PD-L1 or PD-L2, this interaction also can be blocked by anti-PD-L1 or anti-PD-L2 antibodies. These neutralizing antibodies block PD1 signaling and promote T cell activation, resulting in reactivation of the NFAT-responsive luciferase reporter.

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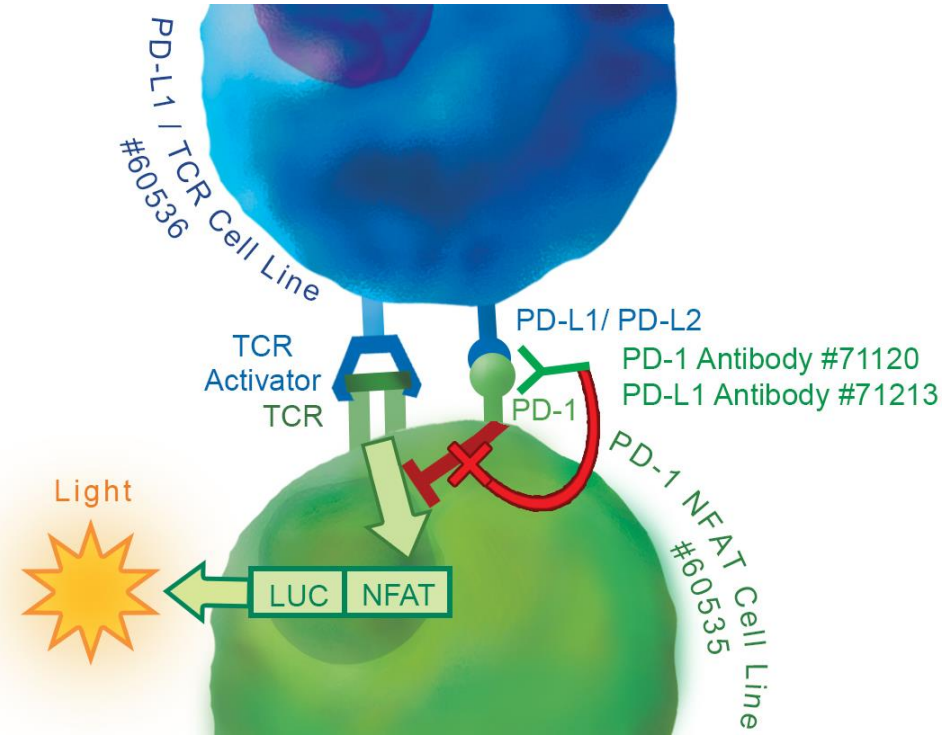

Protocol

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. The next day, transfect 1 µl of Expression vectors for TCR activator and human PD-L1 or PD-L2 into cells following the manufacturer's protocol**.**

3. One day after transfection, quickly thaw the growth-arrested PD-1 Effector 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. Spin down the cells at 1500 rpm, remove supernatant and re-suspend cells in 7 ml of pre-warmed thaw medium.

   To test the anti-PD-1 antibody, prepare serial dilution of the PD-1 antibody in thaw medium (the dilution concentration is 2x the final antibody concentration). Pre-incubate the growth-arrested PD-1 Effector cells with the diluted anti-PD-1 antibody (1:1 volume ratio) for 15-30 minutes. After the pre-incubation step, add the mixture of the Effector cells and antibody to the transfected HEK293 cells (50 µl / well).

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Note: This kit can also be used with anti-PD-L1 or anti-PD-L2 antibodies (available separately). To test the anti-PD-L1 or PD-L2 antibody, prepare serial dilution of the PD-L1 or PD-L2 antibody in thaw medium (the dilution concentration is 2x the final antibody concentration). Remove the medium from the transfected HEK293. Next, pre-incubate the anti-PD-L1/2 antibody (50 µl / well) with transfected HEK293 for 15-30 minutes. Finally, add 50 µl of growth-arrested PD-1 Effector cells to the transfected HEK293 cells.

4. After ~16 hours, perform luciferase assay using the ONE-Step™ luciferase assay system following BPS Bioscience's protocol (http://bpsbioscience.com/media/wysiwyg/60690-1_Luciferase_Reagent_151130.pdf). Briefly, thaw Luciferase Reagent Buffer (Component A) at room temperature and mix well before use. Immediately prior to performing luciferase assay, prepare the luciferase assay working solution by diluting Luciferase Reagent Substrate (Component B) into Luciferase Reagent Buffer (Component A) at a 1:100 ratio and mix well. Prepare only enough for the experiment, remaining Component A and Component B should be stored separately at -20°C.

5. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer.

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 1. Dose response curve of anti-PD-1 neutralizing antibody in PD-1:PD-L1 cell-based assay
HEK293 cells were transiently transfected with human PD-L1 and an engineered T cell receptor (TCR) activator. The next day, growth-arrested PD-1 Effector cells were pre-incubated with anti-PD-1 neutralizing antibody (BPS Cat. #71120) for 30 minutes prior to co-culture with transfected HEK293 cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent 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.

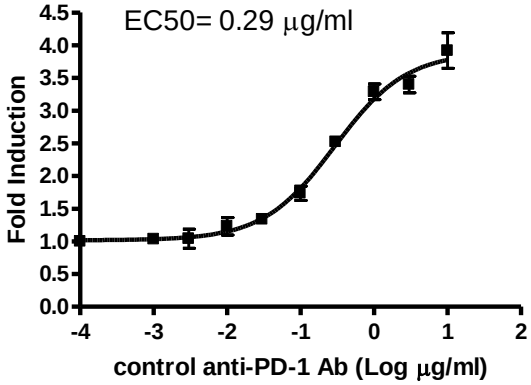

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Figure 2. Dose response curve of anti-PD-L1 neutralizing antibody in PD-1:PD-L1 cell-based assay
HEK293 cells were transiently transfected with human PD-L1 and an engineered T cell receptor (TCR) activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-L1 neutralizing antibody (BPS Cat. #71213) for 30 minutes prior to co-culture with growth-arrested PD-1 effector cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent was added to cells to measure NFAT activity.

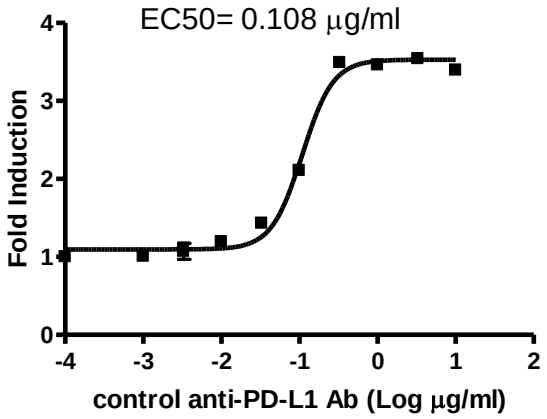
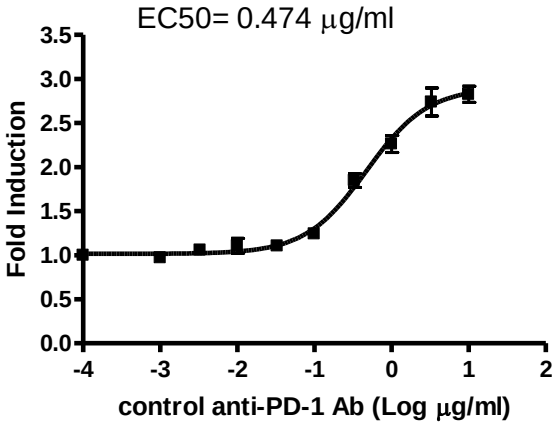


Figure 3. Dose response curve of anti-PD-1 neutralizing antibody in PD-1:PD-L2 cell-based assay
HEK293 cells were transiently transfected with human PD-L2 and an engineered T cell receptor (TCR) activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-1 neutralizing antibody (BPS Cat. #71120) for 30 minutes prior to co-culture with growth-arrested PD-1 effector cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent was added to cells to measure NFAT activity.



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Related Products

Product	**Cat. #**
PD-1/NFAT Reporter - Jurkat cell line	60535
NFAT Reporter – Jurkat cell line	60621
TCR activator / PD-L1 - CHO Recombinant Cell line	60536
TCR activator - CHO Recombinant 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
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