Description

Growth Arrested PD-1 / NFAT Reporter Jurkat Cell Line is a Jurkat T cell cell line expressing firefly luciferase under the control of NFAT response elements with constitutive expression of human PD-1 (Programmed Cell Death 1, PDCD1, SLEB2, CD279, GenBank Accession #NM_005018).

Note: These cells are unable to complete mitosis and are suitable for single use assays. For proliferating cells, please use our PD-1 / NFAT Reporter Jurkat Cell Line (BPS Bioscience #60535).

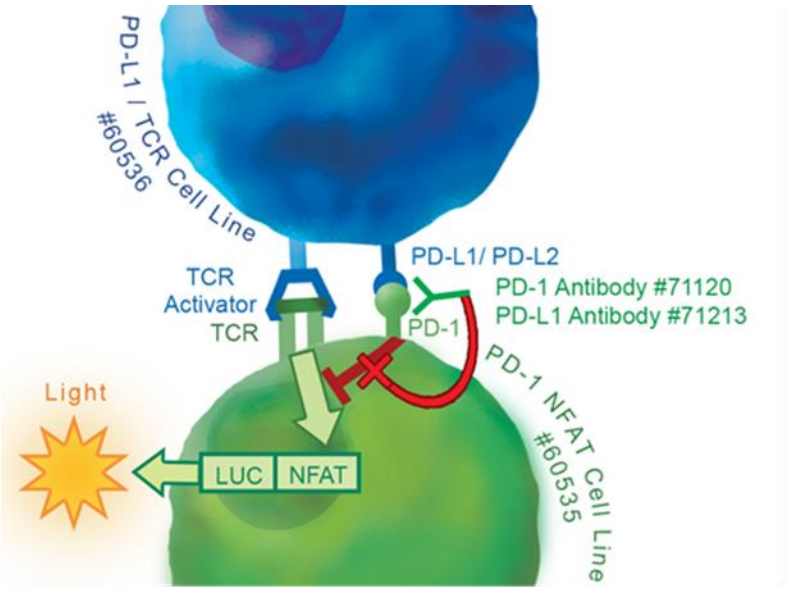


Figure 1: Illustration of the mechanism of action of Growth Arrested PD-1 / NFAT Reporter Jurkat Cell Line in a co-culture assay.

The TCR activator present at the surface of PD-L1/TCR Activator CHO cells stimulate TCR in Jurkat T cells, whereas overexpression of PD-L1 on the CHO cell line engages Jurkat PD-1, blocking TCR activation signaling and preventing activation of NFAT. Addition of a neutralizing anti-PD-1 or anti-PD-L1 antibody to the co-culture releases the PD-L1/PD-1 complex and results in TCR activation and increased NFAT activity, which translates into increased luciferase reporter signal.

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 most 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.

The cell cycle control system acts like a timer, or a clock, that sets a fixed amount of time for the cell to spend in each phase of the cell cycle. The four major phases of the mammalian cell cycle are G1, S, G2 and M phases. Cell-cycle arrest means the cell enters quiescent stage, where the cell is no longer able to undergo cell division.

Application

- Screen for activators or inhibitors of PD-1 signaling in a cellular model.
- Characterize the biological activity of PD-1 and interactions with its ligands.

Materials Provided

Components	Format
1 vial of frozen cells	4 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

Jurkat is a human leukemia cell line, Non-adherent T lymphocytes.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184

Materials Required for Cellular Assay

Name	Ordering Information
PD-L1/ TCR Activator CHO Cell Line ***OR*** PD-L2/ TCR Activator - CHO Cell Line	BPS Bioscience #60536 or BPS Bioscience #79632
Thaw Medium 2	BPS Bioscience #60184
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
Luminometer	
Thaw Medium 3	BPS Bioscience #60186

Storage Conditions

Cells will arrive upon 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.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. To formulate a comparable but not BPS validated media, formulation components can be found below.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Media Required for Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Thaw Medium 3 (BPS Bioscience #60186):
Ham's F-12 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cellular Assay Protocol

This co-culture assay is designed to analyze the effect of PD-L1/PD-1 interaction on Jurkat T cell activation.

- **Conditions should be tested in triplcate.**
- **The assay should have a "Cell-Free Control".**

A. Test of Anti-PD-1 Antibody on Growth Arrested PD-1 / NFAT Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells

1. Harvest PD-L1/TCR Activator CHO cells from culture and seed cells at a density of 35,000 cells per well in 100 µl of Thaw Medium 3 into white clear-bottom 96-well microplate.

2. Incubate cells at 37°C in a CO_2 incubator overnight.

   Note: Cells should reach ~80% confluency on the next day (cells should not reach full confluency in this step).

3. Prepare a serial dilution of anti-PD-1 antibody in Thaw Medium 2 at 2x the final treatment concentration (50 µl/well).

4. Quickly thaw Growth Arrested PD-1 / NFAT Reporter Jurkat cells:

   4.1 Transfer the vial from liquid nitrogen and swirl for 30-40 seconds in a 37°C water-bath, then transfer the vial to a tissue culture hood.

   4.2 Add pre-warmed Thaw Medium 2 to the vial, then the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2.

   4.3 Spin down the cells at 1500 rpm, remove supernatant and re-suspend cells in 7 ml of pre-warmed Thaw Medium 2 (cell count of ~5.5 x 10^5/ml) (50 µl/well).

5. Preincubate the PD-1/NFAT Reporter Jurkat cells (5.5 x 10^5/ml) with diluted anti-PD-1 antibody (1:1 in volume) for 30 min.

6. Remove the medium from PD-L1-/TCR Activator CHO cells and add 100 µl of the 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 thoroughly before adding to the CHO cells.*

7. Add 100 µl of Thaw Medium 2 to the "Cell-Free Control" wells (for determining background luminescence).

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

9. Add 100 µl of ONE-Step™ Luciferase reagent per well.

10. Rock gently at room temperature for ~30 minutes.

11. Measure luminescence using a luminometer.

12. 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.

B. Test of Anti-PD-L1 Antibody on Growth Arrested PD-1 / NFAT Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells

1. Harvest PD-L1/TCR Activator CHO cells from culture and seed cells at a density of 35,000 cells per well in 100 µl of Thaw Medium 3 into white clear-bottom 96-well microplate.

2. Incubate cells at 37°C in a CO_2 incubator overnight.

   Note: Cells should reach ~80% confluency on the next day (cells should not reach full confluency in this step).

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

4. Quickly thaw Growth Arrested PD-1 / NFAT Reporter Jurkat cells:

   4.4 Transfer the vial from liquid nitrogen and swirl for 30-40 seconds in a 37°C water-bath, then transfer the vial to a tissue culture hood.

   4.5 Add pre-warmed Thaw Medium 2 to the vial, then the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2.

   4.6 Spin down the cells at 1500 rpm, remove supernatant and re-suspend cells in 7 ml of pre-warmed Thaw Medium 2 (cell count of ~5.5 x 10^5/ml) (50 µl/ well).

5. Remove the medium from PD-L1/TCR Activator CHO cells and add 50 µl of diluted anti-PD-L1 antibody to the wells and incubate for 30 min.

6. After incubation, add 50 µl of PD-1/NFAT Reporter Jurkat cells (~5.5 x 10^5/ml) to the wells.

   Note: *Mix the PD-1/NFAT Reporter- Jurkat cells thoroughly before adding to TCR activator/PD-L1-CHO cells.*

7. Add 100 µl of Thaw Medium 2 to the "Cell-Free Control" wells (for determining background luminescence).

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

9. Add 100 µl of ONE-Step™ Luciferase reagent per well.

10. Rock gently at room temperature for ~30 minutes.

11. Measure luminescence using a luminometer.

12. 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.

Validation Data

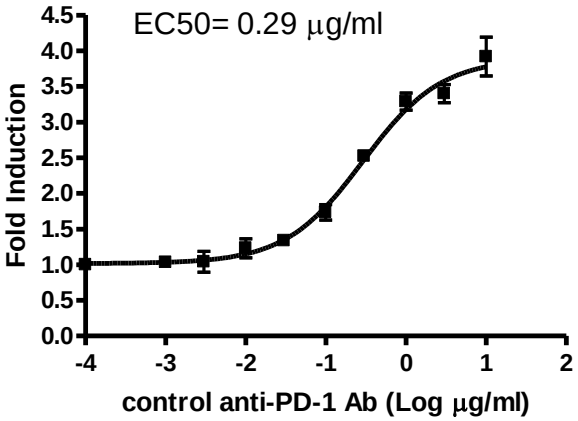


Figure 2. Dose response curve of Growth Arrested PD-1 / NFAT Reporter Jurkat cells to anti-PD-1 neutralizing antibody in PD-1:PD-L1 cell-based assay.
HEK293 cells were transiently transfected with human PD-L1 and an engineered T cell receptor (TCR) activator. The next day, Growth Arrested PD-1 / NFAT Reporter Jurkat cells were pre-incubated with anti-PD-1 neutralizing antibody for 30 minutes, followed by co-culture with transfected HEK293 cells. After ~16 hours of stimulation, NFAT activity was measured with ONE-Step™ Luciferase Assay System. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells.

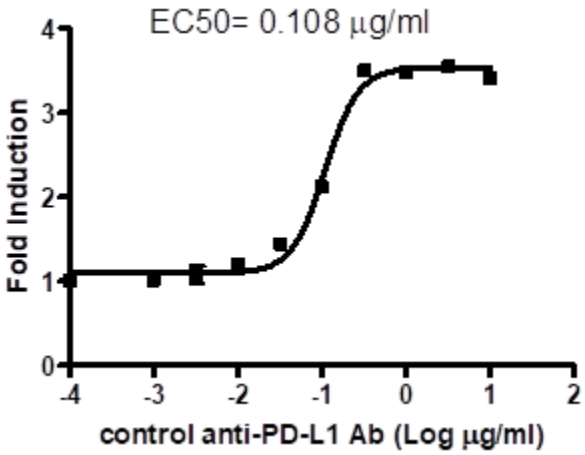


Figure 3. Dose response curve of Growth Arrested PD-1 / NFAT Reporter Jurkat Cell Line to the anti-PD-L1 neutralizing antibody in PD-1:PD-L1 cell-based assay.
HEK293 cells were transiently transfected with human PD-L1 and an engineered T cell receptor (TCR) activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-L1 neutralizing antibody for 30 minutes prior to co-culture with Growth Arrested PD-1 / NFAT Reporter Jurkat cells. After ~16 hours of stimulation, NFAT activity was measured with ONE-Step™ Luciferase Assay System. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells.

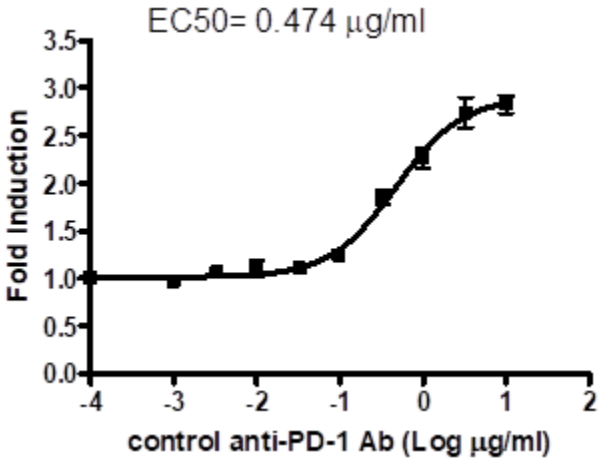
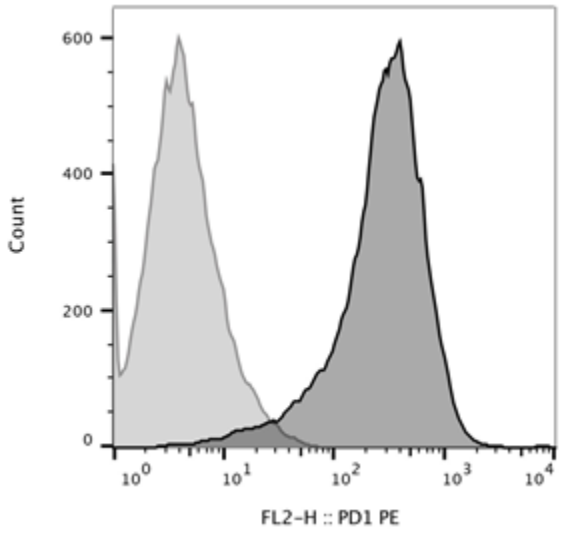


Figure 4. Dose response curve of Growth Arrested PD-1 / NFAT Reporter Jurkat Cell Line to an anti-PD-1 neutralizing antibody in PD-1:PD-L2 cell-based assay.
HEK293 cells were transiently transfected with human PD-L2 and an engineered T cell receptor (TCR) activator. The next day, transfected HEK293 cells were pre-incubated with anti-PD-1 neutralizing antibody for 30 minutes prior to co-culture with Growth Arrested PD-1 / NFAT Reporter Jurkat cells. After ~16 hours of stimulation, NFAT activity was measured with ONE-Step™ Luciferase Assay System. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells.



	Samples	Cell Count
	NFAT reporter-Jurkat	28165
	PD-1/NFAT-Jurkat	27005

Figure 5: PD-1 expression analysis in the PD-1/NFAT Reporter Jurkat Cell Line.
PD-1 / NFAT Reporter Jurkat cells (dark grey) and control NFAT Reporter Jurkat Cell Line (BPS Bioscience #60621, light grey) were stained with anti-PD-1 antibody ana analyzed by flow cytometry.

Sequence
hPD-1 sequence (accession number NM_005018)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAA
FPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQF
QTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATI
VFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

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*
NFAT Reporter – Jurkat cell line	60621	2 vials
Anti-PD-1 Antibody, PE-labeled	71290	50 µg/100 µg
Human PD-1 (CD279), Fc fusion	71106	100 µg/1 mg
Human PD-L1 (CD274), Fc fusion	71104	50 µg/100 µg/1 mg
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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