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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

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

PD-1/IL-2 Luciferase Reporter Jurkat Cell Line is a Jurkat T cell line constitutively expressing human PD-1 (Programmed Cell Death 1, also known as PDCD1, SLEB2, CD279, GenBank Accession #NM_005018), and conditionally expressing firefly luciferase under the control of a human interleukin-2 (IL-2) promoter. This cell line has been validated by flow cytometry for expression of PD-1, and in co-culture assays that assess the PD-L1/PD-1 interaction on Jurkat T cell activation.

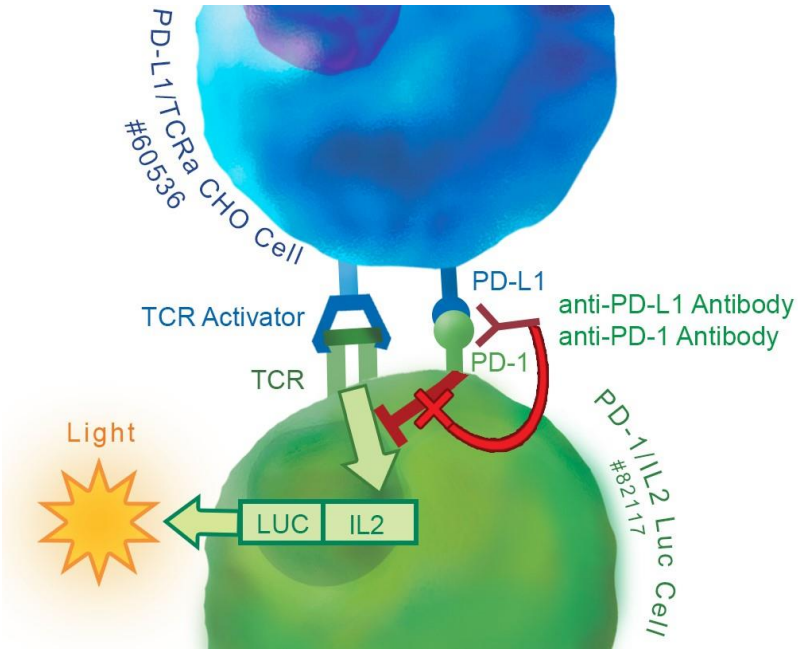


Figure 1: Illustration of the mechanism of action of PD-1/IL-2 Luciferase Reporter Jurkat Cell Line in a co-culture assay.

The TCR activator presented at the surface of PD-L1/TCR Activator CHO cells stimulates TCR (T cell receptor) in Jurkat T cells, whereas overexpression of PD-L1 on the CHO cell line engages Jurkat PD-1 resulting in blocking TCR signaling and preventing activation of the IL-2 promoter. Addition of a neutralizing anti-PD-1 or anti-PD-L1 antibody to the co-culture prevents formation of the PD-L1/PD-1 complex, and results in TCR activation and increased IL-2 promoter 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.

Application

- Screen for inhibitors of PD-1 or PD-L1 signaling in a cellular model.
- Characterize the biological activity of PD-1 and interaction with its ligands.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line
Jurkat (clone E6-1), human T lymphoblast, suspension

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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2A	BPS Bioscience #60190

Materials Required for Cellular Assay

Name	Ordering Information
PD-L1/TCR Activator CHO Cell Line	BPS Bioscience #60536
IL-2 Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60481
Thaw Medium 3	BPS Bioscience #60186
Anti-PD-1 Neutralizing Antibody	BPS Bioscience #71120
Anti-PD-L1 (CD274) Neutralizing Antibody	BPS Bioscience #71213
Anti-CD28 Agonist Antibody (Humanized)	BPS Bioscience #100186
Nivolumab	Selleckchem #A2002
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white clear-bottom assay plate	
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use an -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*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

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

Growth Medium 2A (BPS Bioscience #60190):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1 mg/ml of Geneticin, and 200 µg/ml of Hygromycin B

Media Required for Functional 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

Cell Culture Protocol

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw. Warm up Thaw Medium 2 in a 37°C water bath.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 2 to the conical tube containing the cells. Thaw Medium 2 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2.

5. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2A**.**

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, in Growth Medium 2A**.** The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Cellular Assay Protocol

These co-culture assays are designed to analyze the effect of PD-L1/PD-1 interaction on Jurkat T cell activation.

- Conditions should be tested in triplicate.
- The assay should have a "Cell-Free Control".
- Use of IL-2 Luciferase Reporter Jurkat Cell Line (BPS Bioscience #60481) as control is also recommended.

A. Test of Anti-PD-1 Antibody on PD-1/IL-2 Luciferase Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells

1. Seed PD-L1/TCR Activator CHO cells at a density of 20,000 cells per well in 100 µl of Thaw Medium 3 into a white, clear-bottom 96-well microplate. Keep cell-free wells for the determination of background luminescence (blank).

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

3. Prepare a serial dilution of anti-PD-1 antibody in Thaw Medium 2 at 2x the final treatment concentration (45 µl/well needed).

4. Harvest PD-1/IL-2 Luciferase Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of 8 x 10^5/ml (45 µl/well).

5. Preincubate the PD-1/IL-2 Reporter Luciferase Jurkat cells with the diluted anti-PD-1 antibody (1:1 in volume) for 30 minutes at 37°C (final cell density is ~4 x 10^5/ml).

6. Dilute Anti-CD28 Agonist Antibody in Thaw Medium 2 to 10 µg/ml.

7. Remove the medium from PD-L1-/TCR Activator CHO cells and add 90 µl of the PD-1/IL-2 Luciferase Reporter Jurkat cells / anti-PD-1 antibody mixture to the wells.

Note: Mix the PD-1/IL-2 Luciferase Reporter Jurkat cells with antibody thoroughly before adding to the CHO cells.

8. Add 10 µl of the diluted Anti-CD28 Agonist Antibody to the wells.
9. Add 100 µl of Thaw Medium 2 to the "Cell-Free Control" wells (for determining background luminescence).
10. Incubate the plates at 37°C in a 5% CO_2 incubator for 5-6 hours.
11. Add 100 µl of ONE-Step™ Luciferase reagent per well.
12. Rock gently at room temperature for ~10 minutes.
13. Measure luminescence using a luminometer.
14. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

$$Fold\ induction = \frac{luminescence\ treated\ wells - background}{luminescence\ untreated\ well - background}$$

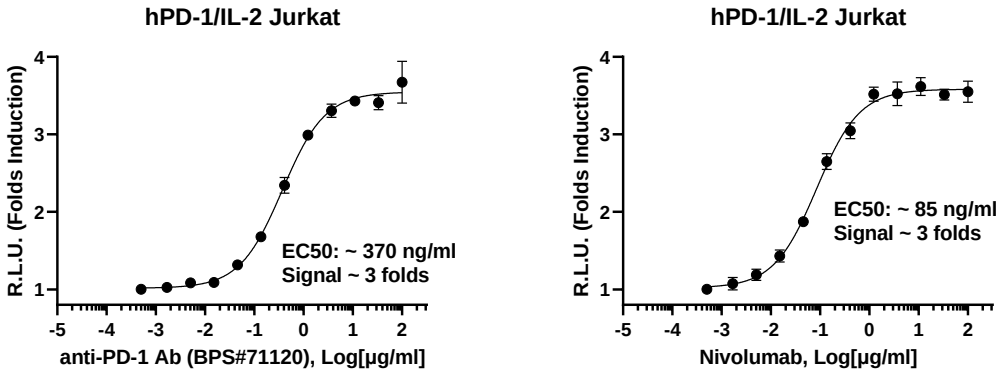


Figure 2. Dose-response curve of PD-1/IL-2 Luciferase Reporter Jurkat Cell Line in response to Anti-PD-1 Neutralizing Antibody and Nivolumab.
A co-culture assay was performed with the PD-L1/TCR-Activator CHO Cell Line as described in the protocol above, in the presence of increasing concentrations of Anti-PD-1 Neutralizing Antibody (left) or Nivolumab (right). Addition of the antibodies to PD-1/IL-2 Luciferase Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells resulted in the dose-dependent activation of IL-2 promoter in Jurkat cells.

B. Test of Anti-PD-L1 Antibody on PD-1/IL-2 Luciferase Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells

1. Seed PD-L1/TCR Activator CHO cells at a density of 20,000 cells per well in 100 µl of Thaw Medium 3 into a white, clear-bottom 96-well microplate. Keep cell-free wells for the determination of background luminescence (blank).

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

3. Prepare a serial dilution of anti-PD-L1 antibody in Thaw Medium 2 at the 2X final treatment concentration (45 µl/well needed).

4. Remove the medium from PD-L1-/TCR Activator CHO cells and add 45 µl of the anti-PD-L1 antibody dilution to the wells.

5. Incubate for 30 minutes at 37°C in a 5% CO_2 incubator.

6. Dilute the Anti-CD28 Agonist Antibody in Thaw Medium 2 to 10 µg/ml.

7. Harvest PD-1/IL-2 Luciferase Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of 8 x 10^5/ml (45 µl/well).

8. Add 45 µl of PD-1/IL-2 Luciferase Reporter Jurkat cells (final cell density ~4 x 10^5/ml) to the wells.

9. Add 10 µl of the diluted Anti-CD28 Agonist Antibody to the wells.

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

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

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

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

14. Measure luminescence using a luminometer.

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

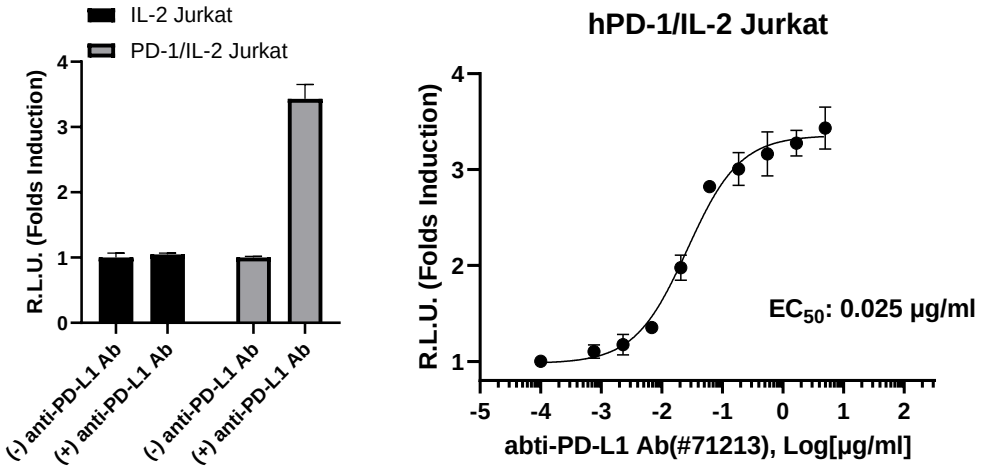
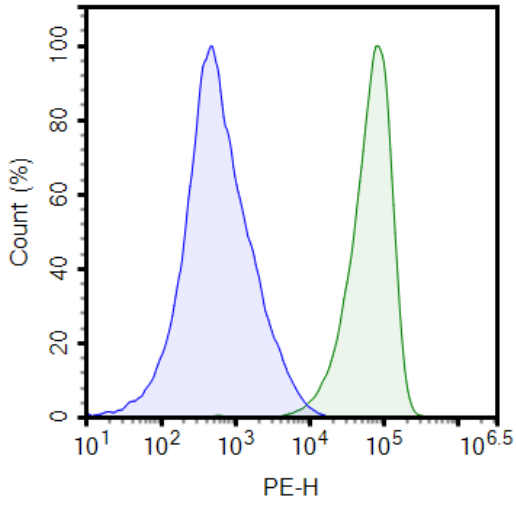


Figure 3. Effect of an anti-PD-L1 Neutralizing Antibody on IL-2 promoter activation in the PD-1/IL-2 Luciferase Reporter Jurkat Cell Line.
A co-culture assay was performed with the PD-L1/TCR-Activator CHO cell line as described in the protocol above. Addition of the anti-PD-L1 neutralizing antibody increased IL-2 promoter-induced luciferase reporter activity in PD-1/IL-2 Luciferase Reporter Jurkat cells, but not in IL-2 Luciferase Reporter Jurkat cells, co-cultured with PD-L1/ TCR Activator CHO cells (left). Addition of increasing concentrations of Anti-PD-L1 antibody to PD-1/IL-2 Luciferase Reporter Jurkat cells co-cultured with PD-L1/TCR Activator CHO cells resulted in the dose-dependent activation of IL-2 promoter in Jurkat cells (right).



F*igure 4. Expression of PD-1 in PD-1/IL-2 Luciferase Reporter Jurkat Cell Line.*
PD-1/IL-2 Luciferase Reporter Jurkat cells (green) or control IL-2 Luciferase Reporter Jurkat cells (blue) were stained with PE-labeled Anti-PD-1 Neutralizing Antibody (BPS Bioscience #71290) and analyzed by flow cytometry. Y-axis represents the % cell number while X-axis indicates the PE intensity.

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

Reading Luminescence
Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry. To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay.

Sequence
Human PD-1 sequence (accession number NM_005018)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAA
FPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQF
QTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATI
VFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

References
Sasca D, *et al.* 2019 *Blood* **133**: 2305-2319

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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*
Anti-PD-1 Neutralizing Antibody, PE-labeled	71290	50 µg/100 µg
PD-1 (CD279), Fc fusion (Human)	71106	100 µg
PD-1, FLAG-Avi-His-tag (Human) HiP™	71198	50 µg
PD-L1 (CD274), Fc fusion (Human) HiP™	71104	50 µg/100 µg
PD-L1 (CD274), FLAG-tag (Human) HiP™	71183	50 µg
PD-L2 (CD273), Fc fusion (Human) HiP™	71107	100 µg