Description

PD-L1 Knockout A549 Cell Line is an A549 lung cancer cell line in which PD-L1 (Programmed Death Ligand 1; CD274; B7 Homolog 1, B7-H1) is no longer expressed due to CRISPR/Cas9 genome editing. This cell line was generated by electroporation of A549 cells with ribonucleoprotein complexes of Cas9 and sgRNAs targeting *CD274*.

This cell line has been validated by genomic sequencing and flow cytometry.

Background

The binding of Programmed Cell Death Protein 1 (PD-1), also known as CD274, a receptor expressed on activated T cells, to its ligands PD-L1 and PD-L2, negatively regulates immune responses. Interferon expression (particularly IFN-γ) from activated T cells induces expression of PD-L1 on tumor cells, which serves as a mechanism of adaptive immune resistance in many cancers. The PD-1 ligands are found on most cancer cells, and the 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 autoimmune diseases such as multiple sclerosis (MS), psoriatic arthritis, lupus, and type I diabetes. The PD-1/PDL-1 pathway is targeted by immune checkpoint inhibitors approved for the treatment of cancer, such as Pembrolizumab (Keytruda®) and Nivolumab (Opdivo®), both of which are used for many types of cancer and were considered blockbuster drugs. Other anti-PD-1 antibodies approved for cancer treatment include Cemiplimab, Dostarlimab, Retifanlimab, Toripalimab, and Tislelizumab. PDL-1-directed checkpoint inhibitors approved for cancer treatment include Atezolizumab, Durvalumab, Avelumab, and Cosibelimab.

Application

- Useful as a control for testing PD-L1-directed immune checkpoint inhibitors and other PD-L1/PD-1 targeted therapies.

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

A549 is a human lung alveolar vessel carcinoma cell line. Adherent epithelial cells.

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 6	BPS Bioscience #60183

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.

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.
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 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol
Note: A549 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 6.

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

2. 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 6.

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

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 6 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Replace media every 2-3 days until cells reach 90% confluency. At first passage and subsequent passages, use Thaw Medium 6.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Thaw Medium 6.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and count the cells.

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

4. 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.

5. 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.

Validation Data

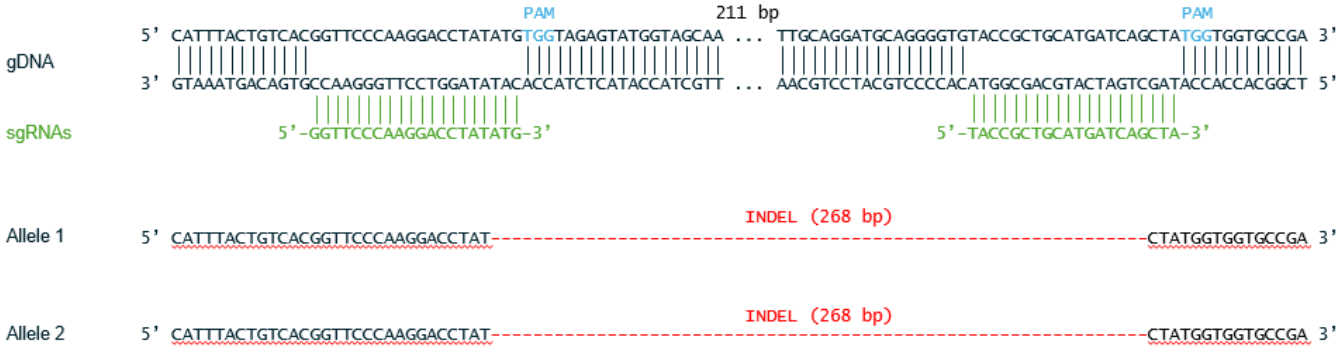

Figure 1. Genomic Sequencing of CD274 (PD-L1) gene, exon 3, in the PD-L1 Knockout A549 Cell Line.
Genomic DNA from the PD-L1 Knockout A549 Cells was isolated and sequenced. The PAMs (Protospacer Adjacent Motifs) are shown in blue, the sgRNAs (single guide RNAs) in green, and the Indel (Insertion/Deletion) in the PD-L1 alleles are indicated in red.

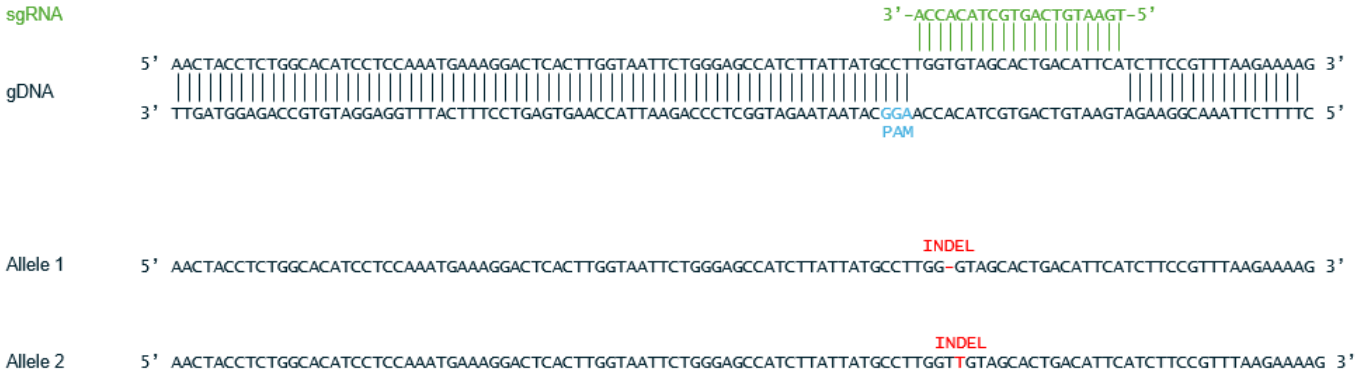

Figure 2. Genomic Sequencing of CD274 (PD-L1) gene, exon 5, in the PD-L1 Knockout A549 Cell Line.
Genomic DNA from the PD-L1 Knockout A549 Cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (single guide RNA) in green, and the Indels (Insertions/Deletions) in the PD-L1 alleles are indicated in red.

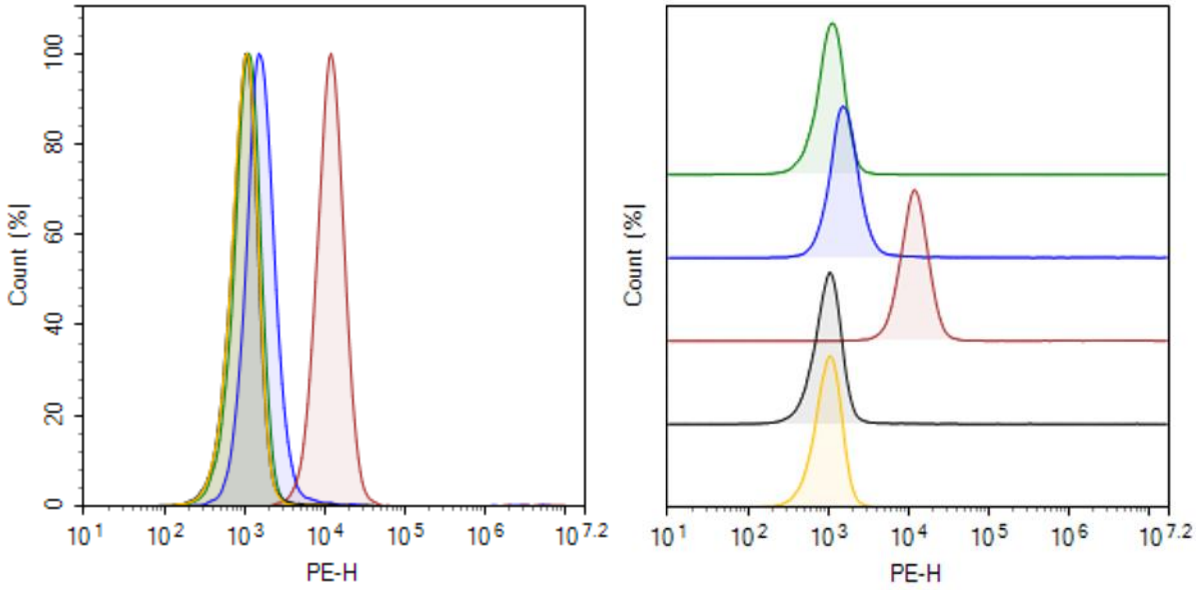

Figure 3. Expression of PD-L1 in the PD-L1 Knockout A549 Cell Line by flow cytometry.
PD-L1 expression can be induced in tumor cells in response to IFN-γ signaling. Cells were cultured in the presence or absence of 100 ng/ml of IFN-γ (#90162) for 48 hours. Cells were then stained with FluoSite Anti-PD-L1 Antibody, PE-Labeled (#102989) and analyzed by flow cytometry. Parental A549 cells (no IFN-γ) are shown in blue, parental A549 cells treated with 100 ng/ml of IFN-γ are shown in red, PD-L1 Knockout A549 cells (no IFN-γ) are shown in gray, and PD-L1 Knockout A549 IFN-γ-treated cells are shown in yellow. Unstained parental A549 cells are shown in green. The y-axis shows the % of cells, while the x-axis represents the fluorophore intensity. PD-L1 expression in parental A549 cells is induced by IFN-γ treatment (red versus blue) but not in PD-L1 Knockout A549 cells (yellow versus grey).

Data shown is representative.

License Disclosure
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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Notes
The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

References
Dong H., *et al.,* 1999 *Nat Med* 5(12):1365-1369.
Garcia-Diaz A., *et al.*, 2017 *Cell Rep* 19(6):1189-1201.
Pardoll, 2012 *Nat Rev Cancer* 12(4):252-264.

Related Products

Products	*Catalog #*	*Size*
FluoSite Anti-PD-L1 Antibody, PE-Labeled	102989	25 or 100 tests
Human Interferon-gamma Recombinant	90162	20 µg or 100 µg
PD-L1 CRISPR/Cas9 Lentivirus (Integrating)	78057	500 µl x 2
PD-L1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78064	500 µl x 2
PD-L1 Lentivirus	78925	500 µl x 2

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