

Description

The PD-1 CHO cell line is a recombinant clonal stable CHO-K1 cell line constitutively expressing human PD-1 receptor (accession number: NM_005018). Surface expression of human PD-1 was confirmed by flow cytometry.

Background

PD-1 (Programmed Death Protein 1, CD279) is a transmembrane protein expressed on the surface of immune cells that binds to PD-L1 or PD-L2, inducing apoptosis of T cells and inhibiting immune responses. Cancer cells often overexpress PD-L1 or PD-L2, allowing them to suppress T cell activation, evade the host immune response, and proliferate. Thus, disrupting the PD-1 co-inhibitory pathways may be an effective approach to developing anti-cancer therapies, as well as other immune diseases like multiple sclerosis, arthritis, lupus, and type I diabetes. The overexpression of PD-1 in this cell line allows for the study of PD-1 antibodies and other novel therapeutics.

Application

- Screen and validate antibodies against PD-1 for drug discovery and research
- Screen for compounds that regulate or inhibit PD-1 in a cellular context
- Optimize and perform biological assays

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2×10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, adherent

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 3	BPS Bioscience #60186
Growth Medium 3B	BPS Bioscience #79529

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO₂. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience #60186):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3B (BPS Bioscience #79529):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin B.

Cell Culture Protocol

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 3 (**no Hygromycin**).
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 3 (**no Hygromycin**).
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 (**no Hygromycin**) and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium **3B (contains Hygromycin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3B and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium **3B (contains Hygromycin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:15 twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3B 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10⁶ cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

- Transfer the vials to liquid nitrogen the next day for storage.



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

Validation Data

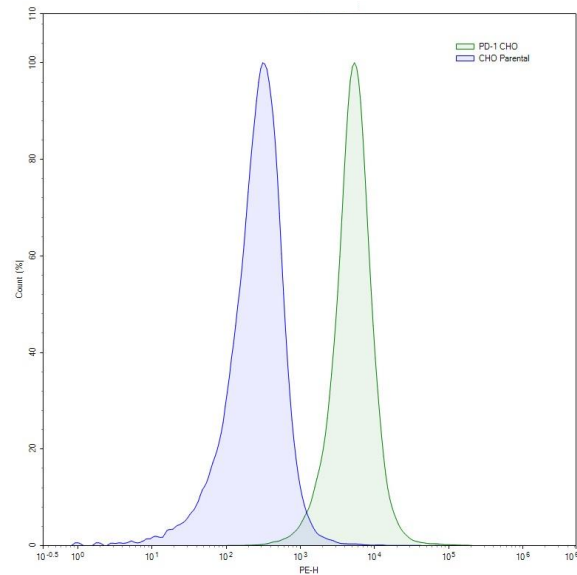


Figure 1: Expression of PD-1 in PD-1 CHO Cell Line.

PD-1 CHO cells (green) or control CHO cells (blue) were stained with PE-labeled Anti-PD-1 Neutralizing Antibody (BPS Bioscience #71290) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Sequence

Human PD-1 sequence (accession number NM_005018)

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MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPPTFSPALLVVTEDGNATFTCSFSNTSESVLWYRMSPSNQTDKLA
FPEDRSQPGQDCRFVRTQLPNGRDFHMSVVRARRRNDSTYLCGAISLAPKAQIKESLRAELRVTTERRAEVPTAHPSPSPR
PAGQFQTLVVGVVGGLLGSLVLLVWVLAVICRAARGTIGARRTGQPLKEDPSAVPVFVSDYGELDFQWREKTPEPPVPCVPEQTEYATI
VFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL*
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References

Han Y, *et al.* (2020) *Am J Cancer Res* 10(3): 727-742

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Anti-PD-1 Neutralizing Antibody, PE-Labeled	71290	100 µg
PD-1 (CD279), Fc fusion (Human) HiP™ Recombinant	71106	100 µg
PD-1 – HEK293 Recombinant Cell Line	60680	2 vials
PD-L1 – CHO Recombinant Cell Line	60543	2 vials
PD-1 / NFAT Reporter – Jurkat Recombinant Cell Line	60535	2 vials
PD-L1 / TCR Activator – CHO Recombinant Cell Line	60536	2 vials