

Description

PD-1 CHO Cell Line is a clonal stable CHO-K1 cell line constitutively expressing human PD-1 receptor (accession number: NM_005018). This cell line has been validated for surface expression of human PD-1 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 model.
- Optimize and perform biological assays.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains $\geq 1 \times 10^6$ 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, 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% CO₂. BPS Bioscience's cell lines are stable for at least 10 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. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.
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 3 to the conical tube containing the cells. Thaw Medium 3 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 3.
5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3B.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca²⁺/Mg²⁺, 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.
3. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3B. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:15 twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS) without $\text{Ca}^{2+}/\text{Mg}^{2+}$, 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 $300 \times g$ for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at $\sim 2 \times 10^6$ 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

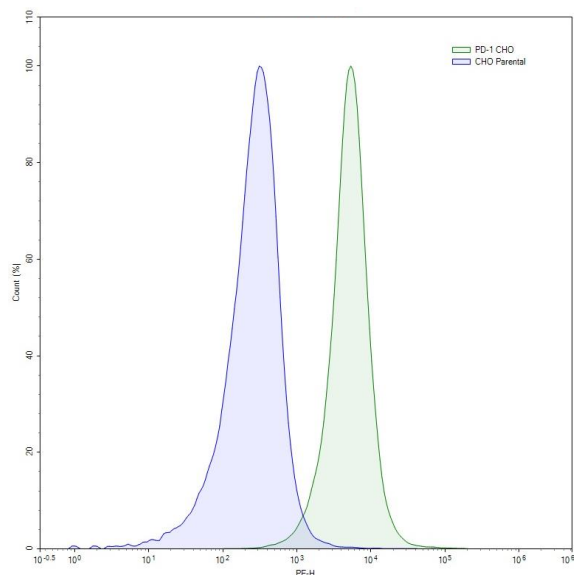


Figure 1: Expression of PD-1 in PD-1 CHO Cell Line.

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

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

Sequence

Human PD-1 sequence (accession number NM_005018)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESVFLNWWYRMSPSNQTDKLAA
 FPEDRSQPGQDCFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPPRPAGQF
 QTLVVGVVGGLLGSLVLLVWVLAVICRAARGTIGARRTGQPLKEDPSAVPVFSDYDYGELDFQWREKTPEPPVPCVPEQTEYATI
 VFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL*

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 Cell Line	60535	2 vials
PD-L1 / TCR Activator CHO Cell Line	60536	2 vials