Description

B7 homolog 3 protein (B7-H3) CHO Cell line is a recombinant clonal stable CHO cell line expressing full length human B7-H3 protein, also known as cluster of differentiation 276 (CD276, B7H3; B7RP-2; 4Ig-B7-H3). Surface expression of B7-H3 was confirmed by flow cytometry. Each stable clonal cell line was selected for High, Medium, or Low levels of B7-H3 expression to mimic different B7-H3 expression levels in various cancer types.

Background

B7-H3 belongs to the B7 family, a group of structurally related cell surface protein ligands that bind to CD28 receptors on lymphocytes to regulate immune response. It is overexpressed in many cancers, acting as a negative regulator of T cell immune responses, thereby aiding in immune evasion. Its expression is associated with poor outcomes and survival times in patients. Through various studies, B7-H3 has been linked to other functions associated with tumor progression, such as angiogenesis, metastasis, and exosomal activity. B7-H3 can also be released in soluble form (sB7-H3) into the tumor microenvironment, where it has been implicated in increased migration and invasion of cancer cells. Due to its relative absence in normal tissues and its multifaceted effects on tumor cells, B7-H3 is an attractive target for immunotherapy treatments.

Application(s)

- Screen B7-H3 antibodies
- Optimize and perform biological assays in a cellular context

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 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% CO_2. 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, and 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% CO_2 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% CO_2 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 1:12 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 10^6 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.

5. 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

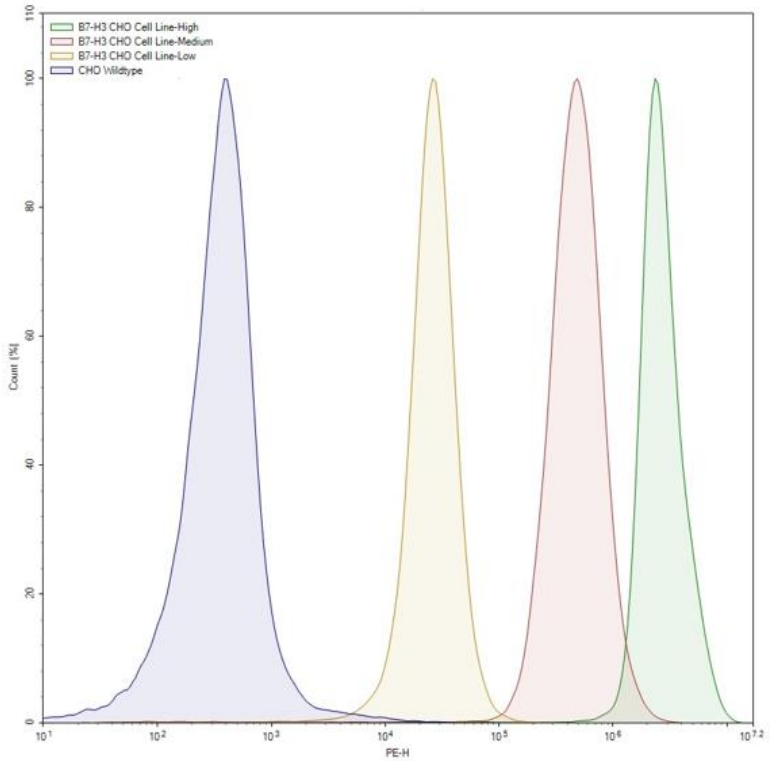

Figure 1: Flow Cytometry analysis of cell surface expression of Human B7-H3 in CHO Cells.
Low, Medium, and High expression B7-H3 CHO Cells (yellow, red, and green, respectively) along with the control CHO cells (blue) were stained with PE-labeled anti-human CD276 (B7-H3) Antibody (Biolegend #351004) and analyzed by flow cytometry. Y-axis represents the % cell Number and X-axis the intensity of PE-H.

Sequence
Human B7-H3 (accession number NM_001024736.2)

MLRRRGSPGMGVHVGAALGALWFCLTGALEVQVPEDPVVALVGTDATLCCSFSPEPGFSLAQLNLIWQLTDTKQLVHSFAEGQ
DQGSAYANRTALFPDLLAQGNASLRLQRVRVADEGSFTCFVSIRDFGSAAVSLQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYQ
GYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTYSCLVRNPVLQQDAHSSVTITPQRSPTGAVEVQV
PEDPVVALVGTDATLRCSFSPEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFPDLLAQGNASLRLQRVRVADEG
SFTCFVSIRDFGSAAVSLQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYRGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLF
DVHSVLRVVLGANGTYSCLVRNPVLQQDAHGSVTITGQPMTFPPEALWVTVGLSVCLIALLVALAFVCWRKIKQSCEEENAGAE
DQDGEGEGSKTALQPLKHSDSKEDDGQEIA*

References

1. Yang, S., Wei, W., & Zhao, Q. Int J Biol Sci. 2020 Mar 25;16(11):1767-1773.
2. Zhou Wu-Tong, Jin Wei-Lin. Front Immunol. 2021 Jul 19;12:701006

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Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
B7-H3, Avi-His-Tag, Biotin-Labeled HiP™ Recombinant	79367	50 µg
B7-H3, Avi-His-Tag HiP™ Recombinant	79337	100 µg
B7-H7 (HHLA2)/TCR Activator CHO Cell Line	78321	2 vials
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials
PD-L1 / TCR Activator - CHO Recombinant Cell Line	60536	2 vials