

**Description**

B7-H4 CHO Cell line is a recombinant clonal stable CHO-K1 cell line constitutively expressing full length human B7-H4 protein, also known as V-set domain-containing T-cell activation inhibitor 1 (VTCN1); (NM\_024626.4). Surface expression of B7-H4 was confirmed by flow cytometry. Each stable clonal cell line was selected for different levels of B7-H4 expression (Low, Medium and High), to mimic different B7-H4 expression levels in various cancer types.

**Background**

B7-H4 is an immune checkpoint that belongs to the B7 family and is encoded by the VTCN1 gene. B7-H4 is expressed on the surface of antigen presenting cells, interacting with currently unidentified ligands to negatively regulate immune cell responses and suppress tumor-associated immunity. B7-H4 also contributes to changes in the tumor microenvironment that benefit cancer cells and support their proliferation, survival, invasion, and metastasis. B7-H4 is highly expressed in some cancer cells, and has been correlated with tumor progression, particularly in ovarian and breast cancer, making it an attractive target for immunotherapy.

**Application**

- Screen and validate antibodies against B7-H4 for drug discovery and research.
- Screen for compounds or ligands that regulate or inhibit B7-H4 signaling in a cellular context.
- Optimize and perform biological assays.

**Materials Provided**

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

**Host Cell**

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.

*Materials Required for Cell Culture*

Name	Ordering Information
Thaw Medium 3	<a href="#">BPS Bioscience #60186</a>
Growth Medium 3B	<a href="#">BPS Bioscience #79529</a>

**Storage Conditions**

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a  $-80^{\circ}\text{C}$  freezer for long term storage. Contact technical support at [support@bpsbioscience.com](mailto: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(s) of interest. Cells should be grown at 37°C with 5% CO<sub>2</sub>. 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, 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.  
**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.
3. Transfer the resuspended cells to a T25 or T75 flask and incubate at 37°C in a 5% CO<sub>2</sub> incubator.
4. Check cell viability after 24 hours in culture. Change medium to fresh Thaw Medium 3 and continue growing in a 5% CO<sub>2</sub> 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.

### Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca<sup>2+</sup>/Mg<sup>2+</sup>, 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.
4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 twice per week.

### Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (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 cell pellet in  $4^\circ\text{C}$  Freezing Medium (BPS Bioscience #79796) at a density of  $\sim 2 \times 10^6$  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  $-80^\circ\text{C}$  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

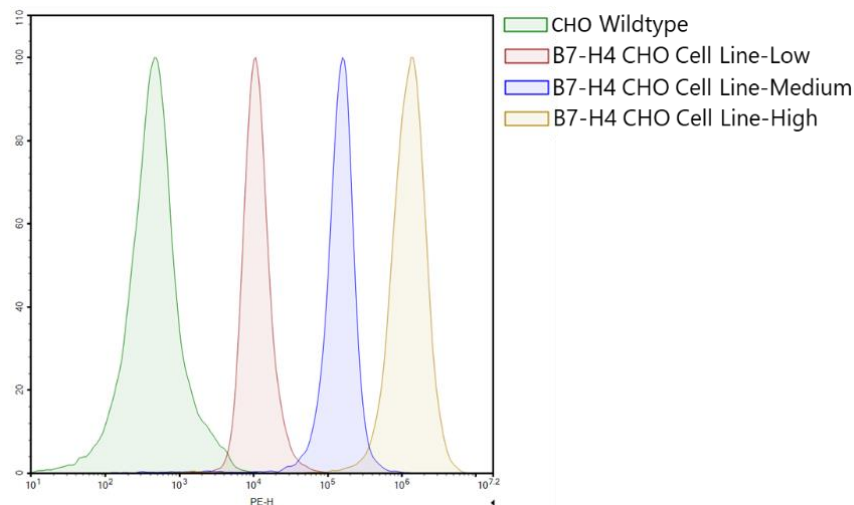


Figure 1. Flow Cytometry analysis of cell surface expression of Human B7-H4 in CHO-K1 Cell lines (low, medium, and high expression).

B7-H4 CHO Cells Low (red,) Medium (blue) and High (yellow) expression, and control CHO cells (green) were stained with PE anti-human B7-H4 Antibody (Biolegend #358104) and analyzed by flow cytometry. Y-axis represents the % cell number. The X-axis represents the intensity of PE-H.

### Sequence

Human B7-H4 (accession number NP\_078902.2)

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MASLGQILFWSIISIIILAGAIALIGFGISGRHSITVTTVASAGNIGEDGILSCTFEPDIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQ
DEMFRGRTAVFADQVIVGNASRLKKNVQLTDAGTYKCYIITSKGKGNANLEYKTGAFSMPEVNVVDYNASSETLRCEAPRWFPQP
TVVWASQVDQGANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYSCMIENDIAKATGDIKVTSEIKRRSHLQLLNSKASLCVSS
FFAISWALLPLSPYLMLK*
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**Troubleshooting Guide**

Visit [bpsbioscience.com/cell-line-faq](https://bpsbioscience.com/cell-line-faq) for detailed troubleshooting instructions. For further questions, email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

**References**

1. Wang JY & Wang WP, 2020, *Cellular immunology* 347: 104008.
2. He C, *et al.*, 2011, *Journal of Immunology Research* 2011: 695834
3. Iizuka A, *et al.*, 2016, *Oncology Reports* 36: 2625-2632.

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
B7-H4, His-Tag, Biotin-Labeled (Human) HiP™ Recombinant	71129	25 µg/ 50 µg
B7-H4 Lentivirus	78727	500 µl x 2
B7-H4, His-Tag (Human) Recombinant	71144	100 µg
B7-H7 (HHLA2)/TCR Activator CHO Cell Line	78321	2 vials