

**Description**

Recombinant clonal CHO cell line stably expressing full-length human claudin-6 (CLDN6 gene accession number NM\_021195.5). Surface expression of claudin 6 was confirmed by flow cytometry. The stable clonal cell line was selected for medium levels of claudin 6 expression compared to the parental CHO-K1 cell line.

**Background**

Claudins are integral membrane proteins and major components of tight junction strands which serve as a physical barrier to prevent solutes and water from passing freely through the paracellular space between epithelial or endothelial cell sheets. Claudin-6 is one of the 27 members of the claudin family.

Claudin-6 is widely expressed in various tumors but rarely expressed in healthy adult tissues. This cancer specific expression of claudin-6 makes it a promising target as an antigen for cancer immunotherapy. Several therapeutics that target claudin-6 are currently in development or in clinical trials including bispecific antibodies, monoclonal antibodies, and CAR-T cell therapy products.

**Application(s)**

- Screen and validate antibodies against claudin-6 for drug discovery and research.
- Screen for compounds that regulate or inhibit claudin-6 signaling in a cellular context.
- Perform binding assays to screen for potential claudin-6 ligands.

**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)

**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	<a href="#">BPS Bioscience #60186</a>
Growth Medium 3D	<a href="#">BPS Bioscience #79539</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, 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<sub>2</sub>. 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 3D (BPS Bioscience #79539):*

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin.

## 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 Geneticin**).  
**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 Geneticin**).
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO<sub>2</sub> incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 (**no Geneticin**) 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 3D (**contains Geneticin**).

### 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 3D 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 3D (**contains Geneticin**). Seed into new culture vessels at the desired sub-cultivation ratio of 1:6 to 1:8 weekly or 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 3D 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  $\sim 2 \times 10^6$  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.

#### A. Validation Data

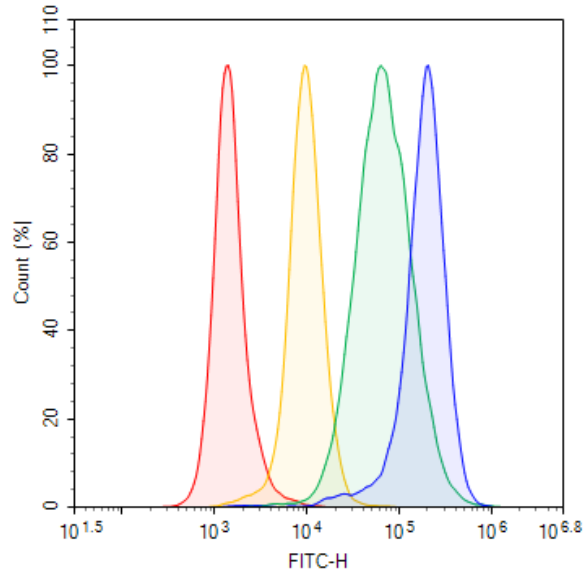


Figure 1: Cell surface expression of Claudin 6 in Claudin-6 CHO Cell Line (Low/Medium/High expression).

One million cells of Claudin-6 CHO cell line (L, M, H) along with control parental CHO-K1 were stained with 0.5  $\mu$ l of Fluor-488 conjugated anti-human claudin-6 antibody (R&D Systems #FAB3656G) for 30 minutes on ice, washed three times, and analyzed by flow cytometry. Claudin-6 CHO low expression (Clone 41, yellow), medium expression (Clone 27, green) and high expression (Clone 8, blue) were compared to the control parental CHO-K1 (red). Y-axis is the % cell count. X-axis is the FITC intensity (the antibody is Fluor-488 conjugated, which has almost an identical excitation and emission spectrum as FITC.)

#### Sequence

> NM\_021195.5 Homo sapiens claudin 6 (CLDN6), mRNA

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MASAGMQILGVVLTLLGWVNGLVSCALPMWKVTAFIGNSIVVAQVWVWGLWMSVCVQSTGQMCKVYDSSLALPQDLQAA
RALCVIALLVALFGLLVYLAGAKCTTCVEEKDSKARLVLTSGIVFVISGVLTLIPVCWTAHAIIIRDFFYNPLVAEAQKRELGASLYLGWA
ASGLLLGGLLCCTCPSGGSQGPSHYMARYSTSAPAIRGPEYPTKNYV
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#### License Disclosure

Visit [bpsbioscience.com/license](https://bpsbioscience.com/license) for the label license and other key information about this product.

#### Troubleshooting Guide

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

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Claudin-18 Isoform 1 CHO Cell Line	78361	2 vials
Claudin-18 Isoform 2 CHO Cell Line (High, Medium, or Low Expression)	78533	2 vials