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Description

Recombinant Claudin-3 CHO K1 cell line stably expressing the full-length human Claudin-3 (accession number: NM_001306). Surface expression of hClaudin-3 was confirmed by flow cytometry. The stable clonal cell line was selected for high level of hClaudin-3 expression compared to the parental CHO K1 cells.

Background

Claudins are cell–cell adhesion proteins expressed in tight junctions (TJs), the most common apical cell-cell adhesion. Claudin proteins regulate defense and barrier functions, as well as differentiation and polarity in epithelial and endothelial cells. Claudins are regulated through interactions with each other that are coordinated with other transmembrane tight junction proteins and cytosolic scaffold proteins. Of the 14 claudins expressed by the alveolar epithelium, claudin-3, claudin-4, and claudin-18 are the most prominent; each confers unique properties to alveolar barrier function. Aberrant expressions of claudins have been reported in various cancers.

Claudin-3 interacts with claudin-1 and claudin-5. It also acts as a low-affinity receptor for Clostridium perfringens enterotoxin (CPE) during infection by gram-positive Clostridial bacteria, which are responsible for food poisoning and gastrointestinal illnesses. Recent studies indicate that the protein may be overexpressed in ovarian cancer. The depletion of claudin-3 increases tumor burden by enhancing β -catenin activity through (IL)-6/STAT3 signaling in colon cancer. Another study identified claudin-3 as a suppressor of lung squamous cell carcinoma cells, as overexpression of claudin-3 inhibited invasion, migration, and EMT of lung squamous cell carcinoma.

Application

- Study antibody-mediated signaling for immunotherapy research and drug discovery
- Screen and characterize Claudin-3 antibodies and ligands

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 3J	BPS Bioscience #79974

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 3J (BPS Bioscience #79974):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 5 µg/ml of puromycin

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 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 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 **3J containing Puromycin**.

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 3J 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 **3J (contains Puromycin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 to 1:20 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 **3J** 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.

Validation Data

Cell surface expression of human Claudin-3 in CHO K1 cells was confirmed by flow cytometry.

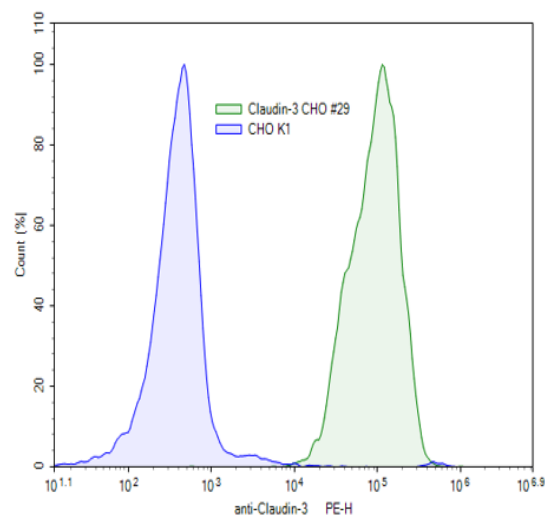


Figure 1. Flow cytometry analysis of cell surface expression of hClaudin-3 in CHO K1 cells. hClaudin-3 CHO K1 cells or control CHO K1 cells were stained with PE-conjugated human Claudin-3 antibody (R&D System #FAB4620P) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Sequence

Human Claudin-3 sequence (accession number NM_001306)

MSMGLIITGTALAVLGLWLTIVCCALPMWRVSAFIGSNIITSQNIWEGLWMNCVVQSTGQMCKVYDSLALPQDLQAARALI
VVAILLAAFGLLVALVGAQCTNCVQDDTAKAKITIVAGVLFLLAALLTLPVSWANTIIIRDFYNPVVPEAQKREMGAGLYVGWA
AAALQLLGGALLCCSCPPREKKYTATKVVSAPRSTGPGASLGTGYDRKDYV

License Disclosure

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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Claudin-4 CHO Cell Line	78566	2 vials
Claudin-3 Lentivirus	78722	500 µl x 2
Claudin-4Lentivirus	78723	500 µl x 2
Claudin-9 Lentivirus	78721	500 µl x 2