

Description

Recombinant clonal CHO cell lines stably expressing full length human claudin 18.2 (claudin18 isoform 2, CLDN18.2, gene accession number NM_001002026.3). Each stable clonal cell line was selected for high, medium, or low levels of claudin 18.2 expression compared to the parental CHO-K1 cell line.

Background

Claudins are integral membrane proteins and 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. The claudin-18 gene has two splice variants: claudin-18.1, which is found primarily in the lung, and claudin-18.2, which is expressed exclusively in the stomach. Claudin-18.2 is highly expressed in gastric and pancreatic adenocarcinoma and may be involved in tumor development and progression. Due to the morphological changes and epithelial-mesenchymal transition occurring upon cancer progression, the extracellular loops of claudin 18.2 become exposed and available for antibody binding. These biological characteristics suggest that it is an ideal target for therapy and have led to the further development of monoclonal antibodies against claudin 18.2, such as Zolbetuximab.

Application(s)

- Test antibodies against claudin 18.2
- Screen inhibitors or neutralizing antibodies against claudin 18.2

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 3D	BPS Bioscience #79539

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 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% CO₂ 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% 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 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 ~2 x 10⁶ 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.

- 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

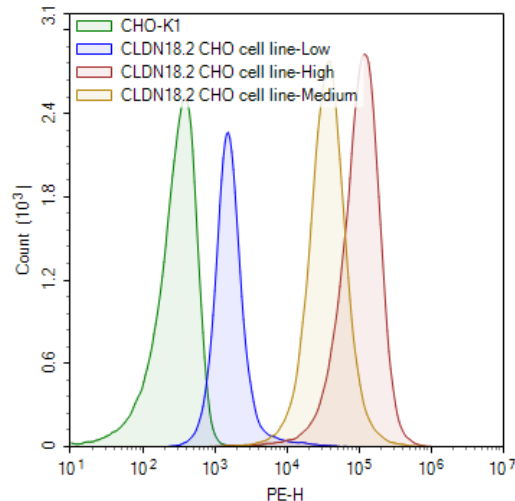


Figure 1. Cell surface expression of Claudin 18.2 in Claudin 18.2 CHO Cell Line.

One million cells were stained with 1-2 μg of biotinylated anti-claudin-18.2 antibody (BPS Bioscience #101565) for 30 minutes followed with 0.2 μg (1:100 dilution) of streptavidin PE conjugate (Thermofisher #12-4317-87), and analyzed by flow cytometry. Claudin-18.2 CHO cells with different expression levels (high expresser: red; medium expresser: yellow; and low expresser: blue) were compared to parental CHO-K1 cells (green). Y-axis is the cell count. X-axis is PE intensity.

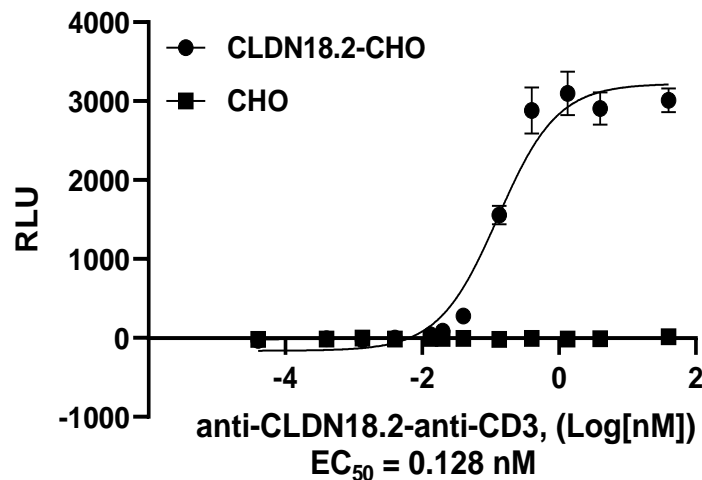


Figure 2: Activation of NFAT Luciferase Reporter Jurkat cells in co-culture with Claudin 18.2 CHO cells.

Approximately 30,000/well NFAT Luciferase Reporter Jurkat cells were added to a culture of Claudin 18.2 CHO cells (BPS Bioscience #78533-H) or to control CHO-K1 cells (ATCC #CCL61™) seeded at 30,000 cells/well in a 96-well plate. Increasing concentrations of anti-CLDN18.2-anti-CD3 bispecific antibody were added to the co-culture and incubated overnight. Luciferase activity in the NFAT Luciferase Reporter Jurkat cells was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Results are expressed as raw luciferase units.

Sequence

NM_001002026.3 Homo sapiens claudin 18 isoform 2 mRNA

MAVTACQQLGFVVSILIGIAGIIAATCMDQWSTQDLYNNPVTAVFNYYQGLWRSCVRESSGFTECRGYFTLLGLPAMLQAVRALM
IVGIVLGAIGLLVSIFALKCIRIGSMEDSAKANMTLTSGIMFIVSGLCAIAGVSVFANMLVTNFWMSTANMYTGMGGMVQTVQT
RYTFGAALFVGWVAGGLTLIGGVMMCIACRGLAPEETNYKAVSYHASGHSVAYKPGGFKASTGFGSNTKNKKIYDGGARTEDEV
QSYPSKHDYV

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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

Products	Catalog #	Size
Claudin-18 Isoform 1 CHO Cell Line	78361	2 Vials
Claudin-6 CHO Cell Line	78527	2 Vials
Anti-Claudin-18 Isoform 2 IgG Antibody, Biotin-Labeled	101565	various
Anti-Claudin-18 Isoform2-Anti-CD3 IgG Bispecific Antibody	101541	various