

Description

Recombinant clonal CHO cell line stably expressing full length human FUT4 (fucosyltransferase 4). Since FUT4 controls the synthesis of antigen CD15, forced expression of FUT4 results in overexpression of CD15 at the cell surface, which was confirmed by flow cytometry. The stable clonal cell line was selected for high levels of CD15 expression compared to the parental CHO-K1 cell line.

Background

FUT4 (fucosyltransferase 4) is an enzyme that attaches fucose to N-acetyllactosamine polysaccharides during the glycosylation process. It is directly involved in the synthesis of the non-sialylated antigen, Lewis x (also known as cluster of differentiation CD15, or SSEA-1), which is attached to O-glycans on the cell surface and plays a role in cell-cell interactions. CD15 is one of the most important blood group antigens and is expressed in several types of immune cells. It functions in leukocyte homing and sperm-egg recognition. It has been implicated in human diseases such as leukocyte adhesion deficiency type 2 and several types of blood cancer, and is believed to play a role in cancer metastasis.

Application

1. Test antibodies against CD15 or FUT4.
2. Screen inhibitors or neutralizing antibodies which disrupt the interaction between CD15 and partner proteins/ligands on other cells.

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 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% 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 3B (BPS Bioscience, #79529):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 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% CO₂ 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% 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 **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 1:6 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 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 $\sim 2 \times 10^6$ 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

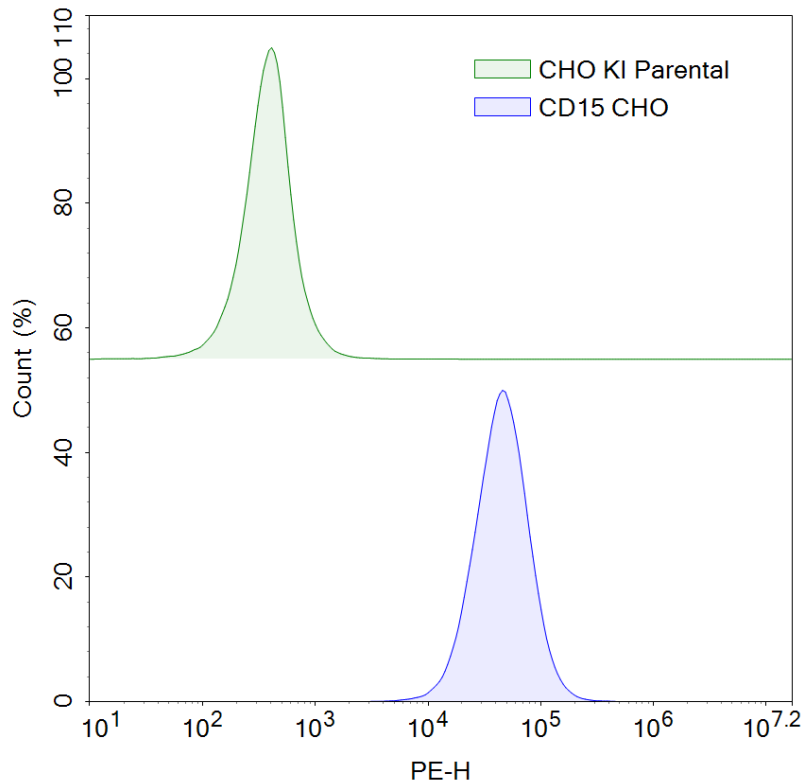


Figure 1. Cell surface expression of CD15 in FUT4-CD15 CHO Cell Line. One million cells were stained with 5 μ l of PE-anti-CD15 antibody (Biolegend, #125606) for 30 minutes on ice, washed three times and analyzed by flow cytometry. FUT4-CD15 CHO cells (blue) were compared to parental CHO-K1 cells (green). Y-axis is the cell count. X-axis is PE intensity.

Sequence

>NP_002024.1 Homo sapiens alpha-(1,3)-fucosyltransferase 4 (FUT4), mRNA

MRRLWGAARKPSGAGWEKEWAEAPQEAPGAWSGRLGPGRSGRKGRAVPGWASWPAHLALAARPARHLGGAGQGPRPLH
SGTAPFHSRASGERQRRLEPQLQHESRCRSSTPADAWRAEAALPVRAMGAPWGSPTAAAGGRRGWRRGRGLPWTVCVLA
GLTCTALITYACWGQLPPLPWASPTPSRPVGVLLWWEFPGGRDSAPRPPDCRLRFNISGCRLLTDASYGEAQAVLFHHRDLV
KGPPDWPPPWGIQAHTAEVDLRVLDYEEAAAAAALATSSPRPPGQRWVWMNFESPSHSPGLRSLASNLFNWTLNADSD
VFVPGYLYPRSHPGDPPSGLAPPLSRKQGLVAWVSHWDERQARVRYHQLSQHVTVDVFRGGPGQPVEIGLLHTVARYK
FYLAFENSQHLDYITEKLWRNALLAGAVPVVLPDRANYERFVPRGAFIHVDDFPSASSLASYLLFLDRNPAVYRRYFHWRRSYAV
HITSFWDEPWCRVCQAVQRAGDRPKSIRNLASWFER

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CD22 / Luciferase - CHO Recombinant Cell Line	79715	2 vials
CD33– CHO K1 Recombinant Cell Line (High, Medium and Low Expression)	79935	2 vials
CD34 CHO Recombinant Cell Line	78336	2 vials
Neuropilin-1, Avi-His-Tag Recombinant	100870	100 µg