

Description

Recombinant clonal CHO cell line stably expressing full length human CD16B (also known as FCGR3B, FcγRIIIb, ref. seq. NM_000570) under the control of the CMV promoter. The surface expression of CD16B was validated by flow cytometry.

Background

The protein encoded by this gene is a low affinity receptor for the Fc region of gamma immunoglobulins (IgG). The encoded protein acts as a monomer and can bind either monomeric or aggregated IgG. This gene may function to capture immune complexes in the peripheral circulation.

Application

Screen antibodies against human CD16B or examine the function of CD16B in a cellular context.

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) |

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.

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): F12-K supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 3J (BPS Bioscience #79974): F12-K supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 5 µg/ml Puromycin.

Cell Culture Protocol

Cell Thawing

- 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 Puromycin**).
Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
- 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 Puromycin**).
- Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
- After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 (**no Puromycin**), and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
- Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3J (**contains Puromycin**).

Cell Passage

- Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
- 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 about 1:10 every 3 days.

Cell Freezing

- Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
- Once the cells have detached, add Growth Medium 3J and count the cells.
- 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.
- 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.

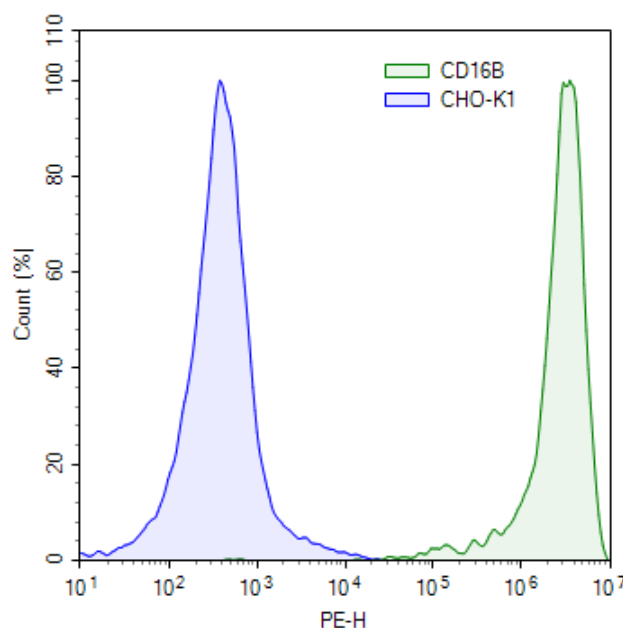
Validation Data

Figure 1. Confirmation of CD16B expression on CHO-K1 recombinant cells

Flow cytometry using PE-conjugated anti-human CD16 antibody (BioLegend #302008) to detect CD16B surface expression on either the CD16B CHO-K1 Recombinant Cell Line (green) or parental CHO-K1 cells (blue).

Sequence

Human FCG3B (CD16B - Sequence Accession Number: NM_000570)

MWQLLLPTALLLVLSAGMRTEDLPKAVVFLEPQWYSVLEKDSVTLKQCQGAYPEDNSTQWFHNENLISSQASSYFIDAATVNDS
GEYRCQTNLSTLSDPVQLEVHIGWLLQAPRWVFKEDPIHLRCHSWKNTALHKVTYLQNGKDRKYFHHNSDFHIPKATLKDSGS
YFCRGLVGSKNVSSETVNITITQGLAVSTISSFSPPGYQVSFCLVMVLLFAVDTGLYFSVKTNI

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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>Product Name</i> | <i>Catalog #</i> | <i>Size</i> |
|--|------------------|-------------|
| FCGR3B (CD16B), Avi-His-Tag | 79016 | 100 µg |
| FcGR3A (CD16A) CHO Cell Line | 78332 | 2 vials |
| FCGR3A (CD16A), Avi-His-Tag | 79013 | 100 µg |
| FCGR3A (F176V), Avi-His-Tag | 100079 | 100 µg |
| Anti-FcGR3A (CD16A) IgG Antibody, Biotin-Labeled | 101225-1 | 50 µg |