

Description

Recombinant CHO cell line stably expressing full length human CD16A (also known as FCGR3A, FcγRIIIa; ref. seq. NM_000569.8) under the control of the CMV promoter.

Background

Fc Gamma Receptor 3A (FcγRIIIA; FcγRIIIA), also known as CD16A, is a low/intermediate affinity receptor for polyvalent immune-complexed IgG. It is involved in phagocytosis, antibody-dependent cytotoxicity, and clearance of immune complexes. The human FcGR3A displays a dimorphism at residue 158. One allele (V158) encodes a higher Fc affinity receptor variant with a valine at amino acid residue 158 while the other allele (F158) encodes a lower Fc affinity receptor variant having a phenylalanine at amino acid residue 158.

FcGR3A (CD16A) plays a role in the activation of natural killer (NK) cells. Clinically, it serves as a marker of certain immune cells such as neutrophils. Its expression has been observed at the surface of T cells in patients with chronic viral infections (such as COVID-19). It is considered a potential therapeutic target to potentiate the efficacy of therapeutic antibodies used to treat solid tumors, or as direct target in hematopoietic cancers.

Application

Screen for antibodies binding to CD16A or examine CD16A function in a cellular context

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2×10^6 cells in 1 ml of 10% DMSO

Host Cell

CHO-K1, 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 this cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents systems 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.

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):

Ham's F-12 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 3J (BPS Bioscience #79974):

Ham's F-12 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin and 5 µg/ml 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 (**no Puromycin**).
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 Puromycin**).
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 Puromycin**), 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 (**contains 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 1:6 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 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 ~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.
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.

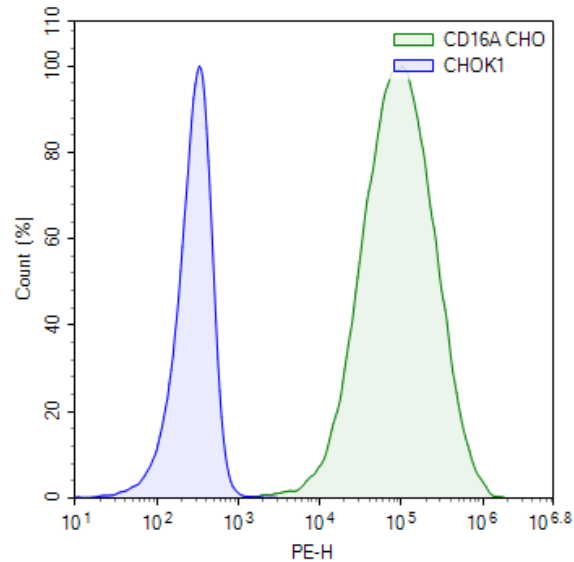
Validation Data

Figure 1. Cell surface expression of FcGR3A (CD16A) in CHO cells. FcGR3A (CD16A) - CHO cells (green) or control CHO cells (blue) were stained with PE-labeled Anti-CD16 Antibody (Biolegend, #980102) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Sequence

FcGR3A (CD16A) sequence (accession number NM_000569.8)

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MWQLLLPTALLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASSYFIDAATVDDSG
EYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSY
FCRGLFGSKNVSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSTRDWKDHKFKWRKDPQDK
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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>
Thaw Medium 3	60186	100 ml/500 ml
Growth Medium 3J	79974	500 ml
ADCC Bioassay Effector Cell F variant (Low Affinity) - Jurkat Recombinant Cell Line	60540	2 vials
ADCC Bioassay Effector Cell F variant (High Affinity) - Jurkat Recombinant Cell Line	60541	2 vials
ADCP Bioassay Effector Cell FcγRIIIa (H variant) /NFAT Reporter-Jurkat	71273	2 vials
FcGR2B- CHO K1 Recombinant Cell Line	79511	2 vials