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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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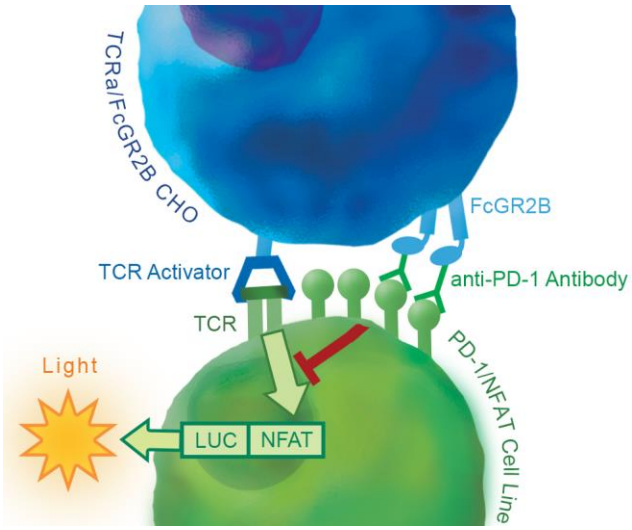
Description

Recombinant clonal CHO-K1 cells stably expressing a membrane bound, engineered TCR (T-cell receptor) activator and human FcGR2B (Fc gamma receptor IIb, NM #004001). The cells were generated from the TCR Activator CHO Cell Line (BPS Bioscience #60539).



Background

FcGR2B (also known as CD32B), is a receptor for the Fc region of immunoglobulin G (IgG) and is known as an immune antibody checkpoint.

The two major forms of FcGR2B, FcGR2B1 and FcGR2B2, arise from either the inclusion or exclusion (respectively) of exon C1 via mRNA splicing, resulting in differing cell type-specific expression and function. Presence of the C1 sequence in isoform 1, which is highly expressed at the surface of B cells, tethers the receptor at the membrane and dramatically increases its half-life at the cell surface. Absence of C1 in isoform 2, expressed in myeloid cells, triggers rapid internalization of the receptor upon ligand binding. FcGR2B induces the phagocytosis of aggregated immunoglobulins. The receptor is also expressed in airways and in liver endothelial cells, where it may act as a "sink" for the removal of IgG immune complexes.

FcGR2B1 operates as a negative regulator of signals induced by antibodies bound to antigens at the surface of cells. Although it acts in concert with dozens of activating receptors, it is the only known negative regulator of B Cell Receptor (BCR)-induced activation of B cells. Thus, the biological function of FcGR2B1 is to tame the antibody-dependent inflammatory response and to clear the circulation of spent immune complexes. Defects in FcGR2B1 signaling lead to overt inflammation and are involved in autoimmune diseases.

In addition, FcGR2B interferes with the efficacy of therapeutic antibodies as it accelerates antibody depletion and decreases B cell responses and antibody production. On the other hand, FcGR2B contributes to the anti-tumor response to antibody checkpoint therapy by boosting CD+ T cells through cross-linking of antibodies directed at stimulatory checkpoints expressed on immune cells such as 4-1BB, OX40, CD40 and GITR.

Therefore, FcGR2B is an important immunotherapy target, both as a direct target for the treatment of B-cell malignancies and in combination with clinically relevant therapeutic monoclonal antibodies to overcome FcGR2B -mediated resistance.

Application(s)

- Screen for regulators of antibody-mediated signaling
- Evaluate the effect of antibodies regulating the regulatory activity of FcGR2B

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 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 3A	BPS Bioscience #60188

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 3	BPS Bioscience #60186
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
PD-1/NFAT Reporter Jurkat cell line	BPS Bioscience #60535
anti-PD-1 IgG antibody	BPS Bioscience #101178
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96 well tissue culture treated white, clear bottom plate	
Luminometer	

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% CO_2. 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 3A (BPS Bioscience, #60188):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1000 µg/ml of Geneticin and 500 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience, #60184):
RPMI-1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Thaw Medium 3 (BPS Bioscience, #60186):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

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 or 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 Geneticin or Hygromycin)**.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 **(no Geneticin or Hygromycin)**, and continue growing in a 5% CO_2 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 3A **(contains Geneticin and 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 3A 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 3A **(contains Geneticin and Hygromycin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:2 to 1:10 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 3A 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 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

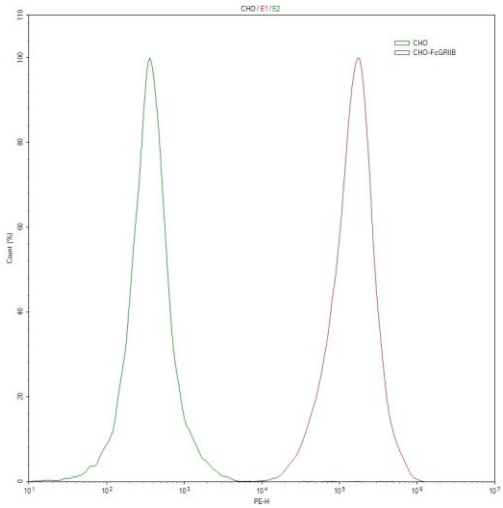


Figure 1: Expression of FcGR2B in TCR Activator/FcGR2B CHO Cell Line. TCR Activator/FcGR2B CHO cells (brown) and TCR Activator CHO cells (BPS Bioscience #60539) (green) were stained with PE-labeled anti-FcGR2B Antibody (Biolegend, #79877) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Functional characterization of FcGR2B/TCR/CHO Cell Line

The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately. Set up each condition at least in triplicate.

Assay Medium:

Thaw Medium 2 (BPS Bioscience, #60184):

RPMI-1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Cellular Assay Protocol

1. Seed the TCR activator/FcGR2B CHO cells at a density of 35,000 cells per well into white clear-bottom 96 well plates in 100 µl of Thaw Medium 3. Leave a few wells empty for background controls. Incubate the cells at 37°C in a CO_2 incubator overnight.

2. Prepare serial dilutions of the test antibodies in Thaw Medium 2 at concentrations 2-fold higher than the desired final concentrations (50 µl/well).

3. Remove the medium from the CHO cells and add 50 ml of antibody dilution per well. Incubate the cells at 37°C in a CO_2 incubator for 30 minutes.

4. Harvest the PD-1/NFAT Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at 400,000 cells per ml of medium.

5. Add 50 ml of PD-1/NFAT reporter Jurkat cells (containing 20,000 Jurkat cells) to the CHO cells pre-treated with the test antibodies

6. Add 100 ml of Thaw Medium 2 to cell-free control wells for determining background luminescence.

7. Incubate the cells at 37°C in a CO_2 incubator for 5-6 hours.

8. After 5-6 hours of incubation, perform the luciferase assay using the ONE-Step™ luciferase assay system (BPS Bioscience #60690): Prepare the ONE-Step reagents as directed and add 100 µl of ONE-Step Luciferase reagent per well. Rock gently at room temperature for ~20 minutes. Measure luminescence using a luminometer.

   Note: *If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.*

9. Data analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NFAT luciferase reporter expression is the background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of untreated control wells.

$$Fold\ induction = \frac{Average\ Lum\ of\ treated\ cells - Average\ background}{Average\ Lum\ of\ control\ cells - Average\ background}$$

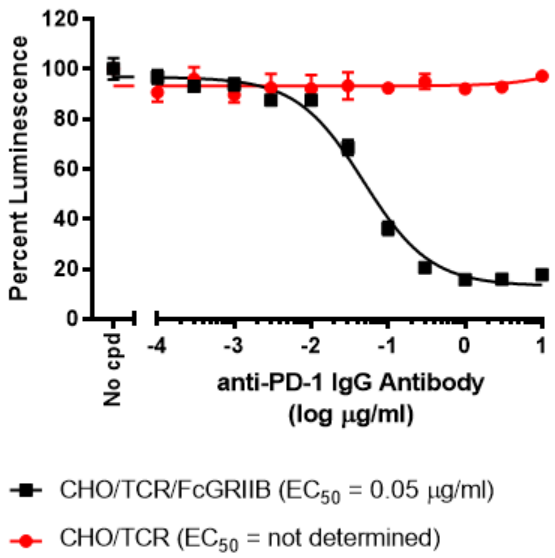


Figure 2. Dose response of anti-PD-1 IgG antibody (BPS Bioscience #101178). A co-culture assay was performed as described above, using the PD-1/NFAT Reporter Jurkat Cell Line (BPS Bioscience #60535) with either the TCR activator/FcGR2B CHO Cell Line or the TCR activator CHO Cell line (BPS Bioscience #60539), in the presence of increasing concentrations of anti-PD-1 antibody (BPS Bioscience #101178). FcGR2B facilitated the agonistic effect of the anti-PD-1 antibody, as indicated by PD-1-mediated inhibition of TCR activation observed in the presence of TCRa/FcGR2B CHO cells, which was not observed in the presence of TRCa CHO cells.

Sequence

Human FcGR2B (GenBank: NM_004001.4)
MGILSFLPVLATESDWADCKSPQPWGHMLLWTAVLFLAPVAGTPAAPPKAVLKLEPQWINVLQEDSVTLTCRGTHSPESDSIQ
WFHNGNLIPTHTQPSYRFKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDKPLVKVTFFQ
NGKSKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQAPSSSPMGIIVAVVTGIAVAAIVAAVVALIYCRKKRISAL
PGYPECREMGETLPEKPANPTNPDEAD KVGAENTITYSLLMHPDALEEPDDQNRI

References

1. Anania JC, *et al.,* The Human FcγRII (CD32) Family of Leukocyte FcR in Health and Disease. *Front Immunol.* (2019) **10: 464**.
2. Teige I, *et al.,* Targeting the Antibody Checkpoints to Enhance Cancer Immunotherapy-Focus on FcγRIIB. *Front Immunol.* (2019) **10: 481**.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq. For all further questions, please email support@bpsbioscience.com.

Related Products

Product	Catalog #	Size
FCGR2A (Human) CRISPR/Cas9 Lentivirus (Integrating)	78207	500 µl x 2
FCGR2A (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78199	500 µl x 2
FcGR2B– CHO K1 Recombinant Cell Line	79511	2 vials
FCGR2A, Avi-His-Tag Recombinant	100094	100 µg
FCGR2B, Avi-His-Tag Recombinant	100089	100 µg
FCGR2B, Avi-His-Tag, Biotin-Labeled Recombinant	100474	25 µg
FCGR2A, Avi-His-Tag Recombinant	100094	50 µg