Description
CHO-K1 cells stably expressing full-length human recombinant FcGR2B (Fc gamma receptor IIb, FcγRIIB). Cell surface expression of human FcGR2B (Genbank #NM_004001.4) was confirmed by flow cytometry.

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
- Characterize the agonist activity of checkpoint antibodies usingFcGR2B receptor-mediated crosslinking

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1mL of 10% DMSO in FBS

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

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Assay Medium: Thaw Medium 3	BPS Bioscience #60186
CD137/NF-κB HEK293 cells	BPS Bioscience #79289
Anti-CD137 recombinant human antibody	BPS Bioscience #79097
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 3D (BPS Bioscience, #79539):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1000 µg/ml of Geneticin

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience, #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 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)**.

   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% CO_2 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% 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 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 (**contains Geneticin)** 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 1:2 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 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 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

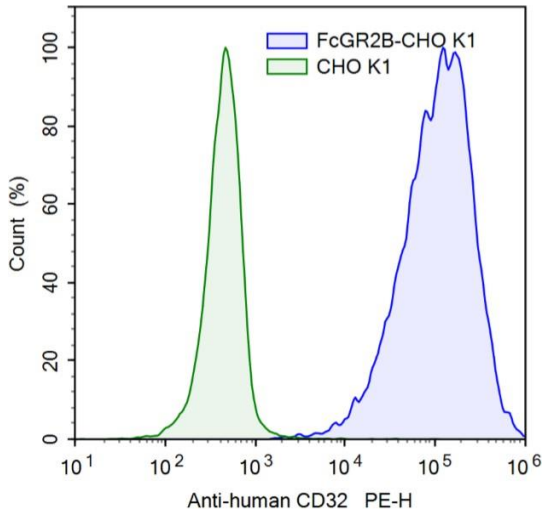

Figure 1: Flow cytometry analysis of cell surface expression of FcGR2B in CHO K1 cells. FcGR2B CHO cells (blue) or parental CHO cells (green) were stained with PE-labeled anti-human CD32 antibody (Biolegend, #303206) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

B. Cellular Assay Protocol

Set up each condition at least in triplicate.

1. Seed CD137/NF-κB reporter HEK293 cells at a density of ~30,000 cells per well into a white clear-bottom 96-well plate in 100 µl of Thaw Medium 1. Leave a couple of wells empty for use as cell-free controls.

2. Incubate the plate at 37°C in a CO_2 incubator overnight. Remove 60 µl of Thaw Medium 1 from each well.

3. 24 hours after seeding, harvest the FcGR2B CHO cells in Thaw Medium 3. Add 50 µl of Thaw medium 3 containing ~90,000 FcGR2B-CHO K1 cells to each well of the CD137/NF-κB HEK293 cells.

3. Prepare serial dilutions of anti- CD137 antibody in Thaw Medium 1 at concentration 10-fold higher than the desired final concentrations (you will need 10 µl/well)

    a. Add 10 µl of diluted anti-CD137 antibody to the treated wells.

    b. Add 10 µl of Thaw Medium 1 to control (untreated) wells.

    c. Add 50 µl of Thaw Medium 1 and 50 ul Thaw Medium 3 to cell-free control wells (for determining background luminescence)

5. Incubate the plate at 37°C in a CO_2 incubator for ~18 hours.

6. Perform the luciferase assay using the ONE-STEP luciferase assay system:

    a. Add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes.

    b. Measure luminescence using a luminometer.

7. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB luciferase reporter expression is the background-subtracted luminescence of stimulated well divided by the average background-subtracted luminescence of unstimulated control wells.

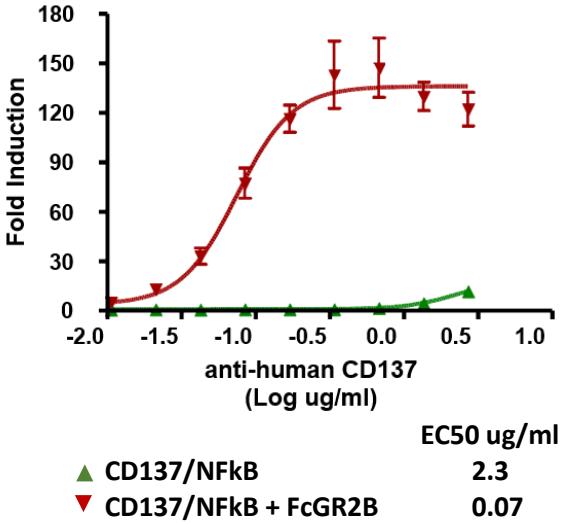

Figure 2: Dose response of anti-CD137 antibody in CD137/NF-κB-reporter HEK293 cells co-cultured with FcGR2B CHO cells. Cross-linking of the anti-CD137 antibody by FcGR2B expressed at the surface of CHO cells allowed activation of NK-κB in CD137-expressing HEK293 cells, indicating that FcGR2B potentiates the efficacy of antibodies directed at checkpoint activator CD137.

Sequence

Human FcGR2B (GenBank: NM_004001.4)

MGILSFLPVLATESDWADCKSPQPWGHMLLWTAVLFLAPVAGTPAAPPKAVLKLEPQWINVLQEDSVTLTCRGTHSPESDSIQWF
HNGNLIPTHTQPSYRFKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDKPLVKVTFFQNGKSK
KFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQAPSSSPMGIIVAVVTGIAVAAIVAAVVALIYCRKKRISALPGYPECRE
MGETLPEKPANPTNPDEAD 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 detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
TCR Activator-FcGR2B CHO Cell Line	78436	2 vials
FcGR2B, Avi-His-Tag Recombinant	100089	100 ug
FcGR2B, Avi-His-Tag, Biotin-Labeled Recombinant	100474	various
FcGR2B (CD32B) Lentivirus	79877	500 µl x2