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Data Sheet

FcGR2B– CHO K1 Recombinant Cell Line
Catalog # 79511

Background

Fc Gamma Receptor 2B (FcGR2B, FcγRIIB), also known as CD32B, is a "low affinity" receptor for Immunoglobulin G (IgG). FcGR2B is involved in the phagocytosis of immune complexes and in the regulation of antibody production by B-cells. Mutations in the gene encoding FcGR2B have been linked to systemic lupus erythematosus (SLE). FcGR2B is the predominant Fc receptor present on B cells, and high expression of FcγRIIB negatively regulates mAb-mediated immunotherapy. Therefore, FcGR2B is an important immunotherapy target, both directly for B-cell malignancies and in combination with clinically relevant therapeutic mAbs to overcome FcGR2B-mediated resistance.

Description

Recombinant FcGR2B-CHO K1 cell line stably expressing full length human FcGR2B (isoform 1, GenBank Accession Number NM_004001.4). Crosslinking of antibodies bound to target by FcGR-expression cells can promote receptor clustering and increase downstream signaling. FcGR2B crosslinking is important for anti-TNFR receptor antibodies.

Application

- Screen for activators or inhibitors of antibody-mediated signaling by coculturing with the FcGR2B CHO K1 cells.
- Characterize the agonist activity of antibodies by crosslinking to the FcGR2B receptor.

Format

Each vial contains ~ 2 x 10^6 cells in 1mL of 10% DMSO in FBS.

Storage

Store in liquid nitrogen immediately upon receipt.

Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

Culture Medium

Thaw Medium 3 (BPS Bioscience, #60186): Ham's F-12 medium (Hyclone, #SH30526.01) supplemented with 10% FBS (Life technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

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Growth Medium 3D (BPS Bioscience, #79539): Thaw Medium 3 (BPS Bioscience, #60186) plus 1 mg/ml Geneticin (G418) (Thermo Fisher, #11811031).

Recommended Culture conditions

Frozen Cells: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw Medium 3. Quickly thaw cells in a 37°C water bath with constant and slow agitation. After cleaning the outside of the vial with 70% ethanol, immediately transfer the entire content to Thaw Medium 3 **(no G418)**. Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. The next day, change to fresh Thaw Medium 3 **(no G418)**. without disturbing the attached cells. Continue to incubate until cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. At first passage, switch to Growth Medium 3D **(contains G418).**

Subculture: When cells reach 90% confluency, remove the medium and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml of prewarmed Growth Medium 3D and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 mL conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and resuspend cells in 10 ml pre-warmed Growth Medium 3D. Dispense 1 mL of the cell suspension into a new T75 flask containing pre-warmed 19 ml Growth Medium 3D (a subcultivation ratio of 1:10 to 1:20 is recommended). Incubate cells in a humidified 37°C incubator with 5% CO_2.

To freeze cells, resuspend cell pellet in freezing medium (10% DMSO in FBS).

Validation

Cell surface expression of human FcGR2B in CHO K1 cells was confirmed by flow cytometry.

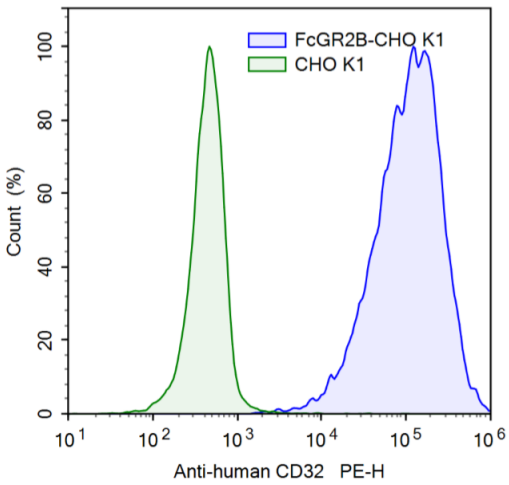

Figure 1. Flow cytometry analysis of cell surface expression of FcGR2B in CHO K1 cells. FcGR2B-CHO K1 cells (blue) or control CHO K1 cells (green) were stained with PE-labeled anti-human CD32 antibody (Biolegend, #303206) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.

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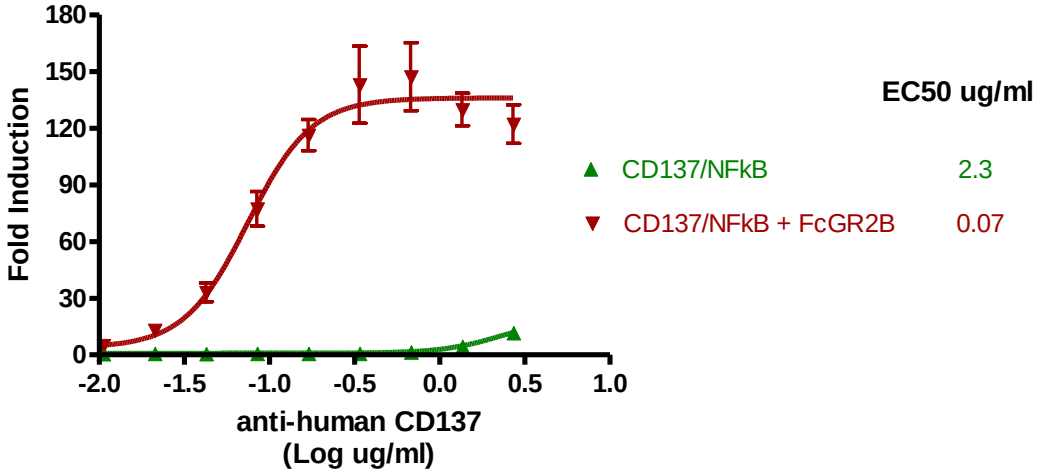

Figure 2. Dose response of anti-CD137 antibody on CD137/ NF-κB-reporter HEK293 cells cocultured with FcGR2B CHO K1 cells.

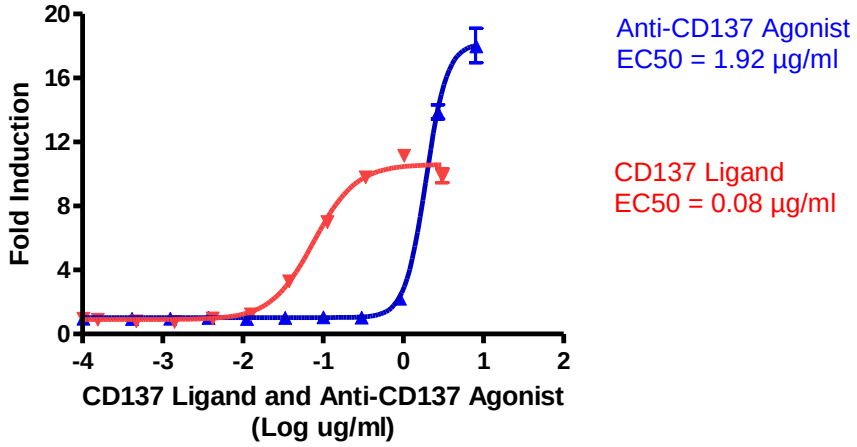

Figure 3. Dose response of CD137/ NF-κB-reporter HEK293 cells. CD137 ligand (BPS Bioscience, #71189) and anti-CD137 agonist (BPS Bioscience, #79097) were diluted and added to the cells (BPS Bioscience, #79289), then incubated at 37°C cell culture incubator for 6 and 24 hrs. respectively. After the treatment, perform Luciferase assay using One-Step Luciferase assay system (BPS Bioscience, #60690).

Assay Protocol for Antibody Crosslinking with FcGR2B-CHO K1 Cells

1. Harvest CD137/NF-κB reporter-HEK293 cells from culture in growth medium and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well

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microplate 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 Thaw medium 1 from each well.

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

4. Dilute the anti-human-CD137 antibody in Thaw Medium 1.

   Add 10 µl of diluted anti-CD137 antibody to the treated wells.
   Add 10 µl Thaw Medium 1 to control wells.
   Add 50 µl of Thaw Medium 1 and 50 ul Thaw Medium 3 to cell-free control wells (for determining background luminescence)

   Set up each treatment in at least triplicate.

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

6. Perform luciferase assay by using the ONE-STEP luciferase assay system, following the recommended protocol. Add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. 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 = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Vector and Sequence
Human FcGR2B (accession number: NM_004001.4) was cloned into pCDNA3.1

MGILSFLPVLATESDWADCKSPQPWGHMLLWTAVLFLAPVAGTPAAPPKAVLKLEPQWINVLQEDSVTLT
CRGTHSPESDSIQWFHNGNLIPTHTQPSYRFKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPH
LEFQEGETIVLRCHSWKDKPLVKVTFFQNGKSKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKP
VTITVQAPSSSPMGIIVAVVTGIAVAAIVAAVVALIYCRKKRISALPGYPECREMGETLPEKPANPTNPDEAD
KVGAENTITYSLLMHPDALEEPDDQNRI

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Related Product	**Cat. #**	**Size**
CD137/NF-κB Reporter - HEK293 Cell Line	79289	2 vials
NF-κB Reporter (Luc)-HEK293 cell line	60650	2 vials
Anti-CD137 Agonist Antibody	79097-1	50 ug
Human CD137L, His-tag	71189	100 ug
CD137 (4-1BB), Fc fusion (Human) HiP™	71170	100 ug
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml