Description

BDCA2 NFAT Luciferase Reporter Jurkat Cell Line is a Jurkat cell line that expresses human BDCA2 (Blood Dendritic Cell Antigen 2), adapter FcεRIγ (Fc epsilon receptor I), and the firefly luciferase reporter under the control of NFAT (Nuclear Factor of Activated T-cells) response elements located upstream of the minimal TATA promoter.

BDCA2 expression was verified by flow cytometry, and this cell line was functionally validated with the anti-BDCA2 antibody Litifilimab in co-culture assays.

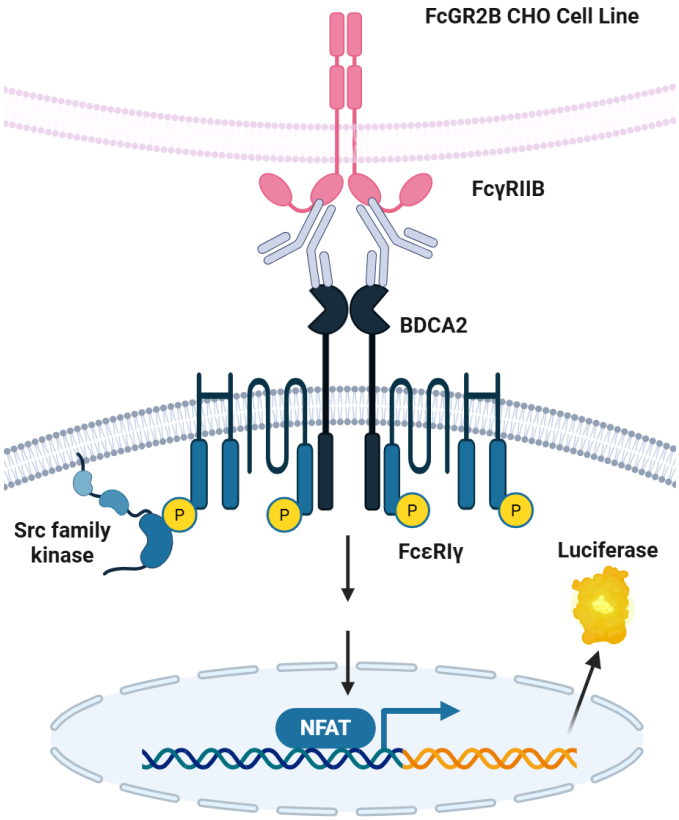

Figure 1: Representation of the mechanism of action of BDCA2 NFAT Luciferase Reporter Jurkat Cell Line in a co-culture assay with FcGR2B CHO Cell Line in the presence of Litifilimab.

Background

Blood Dendritic Cell Antigen 2 (BDCA2), also known as CD303 or CLEC4C, is a type II C-type lectin specifically expressed on human plasmacytoid dendritic cells (pDCs). It is a glycan-binding receptor that inhibits the production of type I interferon. BDCA2 does not contain any signaling motifs, and it triggers signaling through the high-affinity IgE receptor FcεRIγ, inducing a B cell receptor (BCR)-like cascade. Binding to BDCA2 results in inhibition of TLR7 (Toll-like receptor 7) and TLR9-induced production of IFN-I (interferon) and other pro-inflammatory cytokines. IFN-I is linked to SLE (systemic lupus erythematosus), therefore BDCA2 has become an attractive therapeutic target in autoimmunity. Litifilimab, a humanized IgG1 monoclonal antibody targeting BDCA2, was given Breakthrough Therapy Designation by the FDA for the treatment of SLE. Litifilimab binds primarily to BDCA2 but also to FcGR2b, which enhances the inhibitory signal by stabilizing internalization and signal. Further studies into this protein will bring a new understanding of its roles, and new therapies for patients with SLE.

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Application

- Screen for BDCA2 biologics in a cellular model.
- Evaluate the potency of BDCA2-directed candidate drugs.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

Jurkat (clone E6-1), human T lymphoblast, suspension.

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 2	BPS Bioscience #60184
Growth Medium 2F	BPS Bioscience #79669

Materials Required for Cellular Assay

Name	Ordering Information
FcGR2B CHO K1 Cell Line	BPS Bioscience #79511
Litifilimab	BPS Bioscience #83992
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white clear-bottom assay 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.

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Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2F (BPS Bioscience #79669):
RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1 mg/ml of Geneticin, and 0.5 µg/ml of puromycin.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

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

Cell Culture Protocol

Note: Jurkat cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 2.

   Note: 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 2.

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

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2, 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 reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2F**.**

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Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, with Growth Medium 2F**.** The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

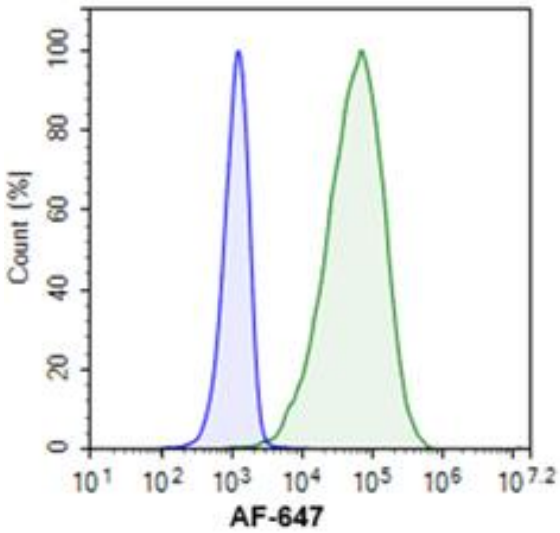

Figure 2. Cell surface expression of BDCA2 in BDCA2 NFAT Luciferase Reporter Jurkat Cell Line analyzed by flow cytometry.
BDCA2 NFAT Luciferase Reporter Jurkat cells (green) and control NFAT Luciferase Reporter Jurkat cells (blue) were stained with Litifilimab, followed by Alexa Fluor® 647 anti-human IgG Fc Antibody and analyzed by flow cytometry. Y-axis represents the cell count. X-axis indicates Alexa Fluor 647 (AF-647) intensity.

Functional Validation

- This co-culture assay is designed to analyze the effect of an BDCA2 antibody on Jurkat cell activation.
- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.

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- The assays should include “Cell-Free Control”, "Untreated Control” and “Treated” conditions.
- The use of NFAT Luciferase Reporter Jurkat Cell Line (#60621) and CHO-K1 cells as controls is recommended.

A. Testing of anti-BDCA2 antibody (Litifilimab) on a co-culture of BDCA2 NFAT Luciferase Reporter Jurkat Cell Line and FcGR2B CHO Cell Line.

1. Seed FcGR2B CHO cells at a density of 20,000 cells per well in 100 µl of Thaw Medium 3 into a white, clear-bottom 96-well cell culture plate. Leave a few wells empty to use as the “Cell-Free Control” (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator overnight.

3. Prepare a serial dilution of anti-BDCA2 antibody in Thaw Medium 2 at 2x the final treatment concentration (50 µl/well).

4. Remove the medium from FcGR2B CHO cells and add 50 µl of the diluted anti-BDCA2 antibody to the “Treated” wells.

5. Add 50 µl of Thaw Medium 2 to the “Untreated Control” wells.

6. Incubate for 30 minutes at 37°C in a 5% CO_2 incubator.

7. Harvest BDCA2 NFAT Luciferase Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of 4 x 10^5 cells/ml (50 µl/well).

8. Add 50 µl of BDCA2 NFAT Luciferase Reporter Jurkat cells (4 x 10^5 cells/ml) to the “Treated” and “Untreated Control” wells.

9. Add 100 µl of Thaw Medium 2 to the “Cell-Free Control” wells (for determining background luminescence).

10. Incubate the plates at 37°C in a 5% CO_2 incubator for 5 hours.

11. Add 100 µl of ONE-Step™ Luciferase reagent per well.

12. Rock gently at RT for ~15 minutes.

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

$$Fold\ induction = \frac{luminescence\ treated\ wells - background}{luminescence\ untreated\ wells - background}$$

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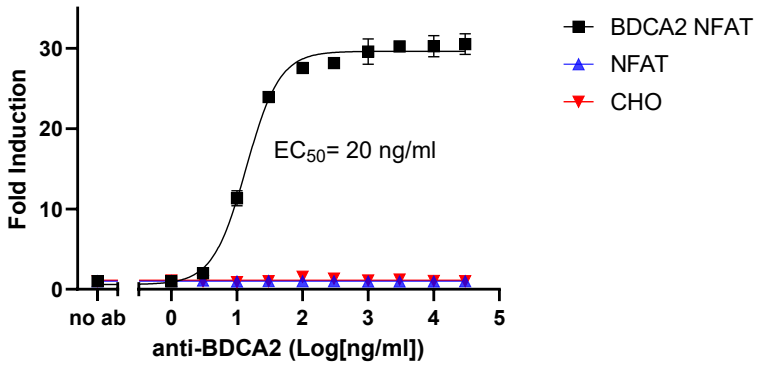

Figure 3. Activation of BDCA2 NFAT Luciferase Reporter Jurkat Cell Line by Anti-BDCA2 Antibody when co-cultured with FcGR2B CHO K1 Cell Line.
BDCA2 NFAT Luciferase Reporter Jurkat cells were co-cultured with FcGR2B CHO cells as described in the protocol above in the presence of increasing concentrations of the anti-BDCA2 antibody Litifilimab. Cross-linking of the BDCA2 antibody by FcGR2B expressed at the surface of CHO cells allowed the activation of NFAT in BDCA2 NFAT Luciferase Reporter Jurkat cells (Black).
As a negative control, CHO cells (red) and NFAT Luciferase Reporter Jurkat cells (blue) were used.
As expected, no activation of NFAT in NFAT Luciferase Reporter Jurkat cells was observed.

Data shown is representative.

Sequence
Human BDCA2 sequence (accession number NP_569708.1)
MVPEEEPQDREKGLWWFQLKVWSMAVVSILLLSVCFTVSSVVPHNFMYSKTVKRLSKLREYQQYHPSLTCVMEGKDIEDWSCC
PTPWTSFQSSCYFISTGMQSWTKSQKNCSVMGADLVVINTREEQDFIIQNLKRNSSYFLGLSDPGGRRHWQWVDQTPYNENV
TFWHSGEPNNLDERCAIINFRSSEEWGWNDIHCHVPQKSICKMKKIYI

References
Cao W., et al., 2007 PLoS 5(10):e248.

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Troubleshooting Guide
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