Description

ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Reporter Jurkat Cells are Jurkat T cells engineered to express firefly luciferase under the control of NFAT response elements, and human FcγRIIa, H variant. This cell line was functionally validated in a ADCP (antibody-dependent cell-mediated phagocytosis) assay.

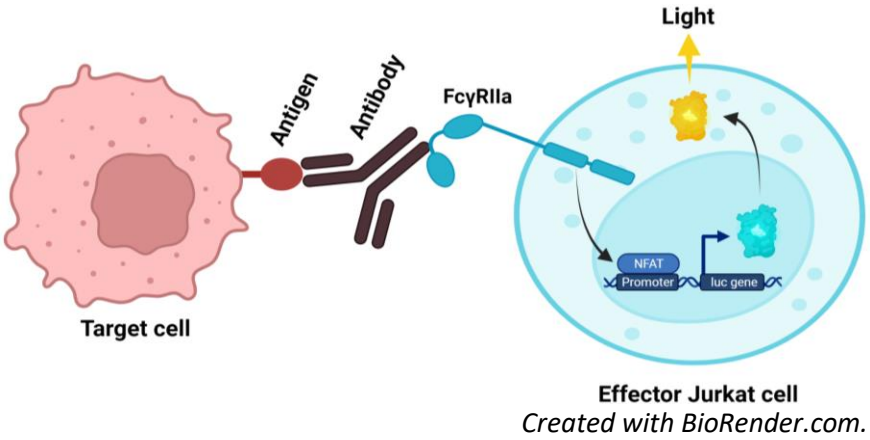

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Figure 1: Illustration of the mechanism of action of ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat Cell Line.

ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat cells are used as effector cells. The effector cells are co-cultured in the presence of target cells and an antibody of interest. The antibody binds to the target antigen on the target cell whereas its Fc portion binds to FcγRIIa on the cell surface of the effector cell, cross-linking the effector and target cells. Engagement of FcγRIIa leads to the activation of the NFAT pathway in the effector cells. Luciferase activity is proportional to the activation of the ADCP cascade.

Background

Antibody-dependent cell-mediated phagocytosis (ADCP) is an important mechanism of action to consider during antibody drug development. FcγRIIa (also known as CD32a) is the predominant Fcγ receptor involved in the ADCP process. FcγRIIa is expressed in myeloid effector cells, including macrophages and neutrophils, where it plays a role in their activation. Engineered amino-acid substitutions in the Fc portion of monoclonal antibodies (mAb) can enhance the mAb-mediated phagocytosis of tumor cells by tumor-associated macrophages (TAMs). It is now clear that one of the major modes of action of therapeutic antibodies, that leads to positive outcomes, is their ability to trigger ADCP in TAMs. The binding of antibodies to Fcγ receptors leads to the phosphorylation of ITAMs (immunoreceptor tyrosine-based activation motifs) and activation of signaling pathways leading to phagocytosis via Rac-GEFs (guanine exchange factors). This mechanism of action has contributed to the success of rituximab (anti-CD20 antibody) in the treatment of chronic lymphocytic leukemia (CCL). Further understanding of this pathway, how to modulate and activate it, will lead to processes in cancer therapy.

Application

- Screen and validate Fc effector function of antibodies in ADCP assays.
- Measure ADCP responses.

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 is a human leukemia cell line, Non-adherent T lymphocytes.

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 2A	BPS Bioscience #60190

Materials Required for Cellular Assays

Name	Ordering Information
Anti-hHER2 human IgG1 Antibody	R&D Systems #MAB9589-SP
Anti-CD20 Functional Antibody	BPS Bioscience #71209
BT-474 Cells	ATCC #HTB-20
Raji Cells	ATCC #CCL-86
Human B Cells WIL2-S	ATCC #CRL-8885
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	BPS Bioscience #60621
96-well tissue culture-treated white clear-bottom assay plate	
One-Step™ Luciferase Assay System	BPS Bioscience #60690
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, 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°C 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):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin, and 200 µg/ml of Hygromycin B.

Assay Medium:
Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 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. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 2 to the conical tube containing the cells. Thaw Medium 2 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

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

6. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of fresh Thaw Medium 2 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they reach 2.5 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2A.

Cell Passage
Dilute cell suspension into new culture vessels with Growth Medium 2A at no less than 0.2 x 10^6 cells/ml. We recommend a sub-cultivation ratio of 1:10 twice a week to maintain the cell density between 0.2 x 10^6 cells/ml to 2.5 x 10^6 cells/ml.

Note: Just after thawing, the cells may grow at a slower rate. It is recommended to split the cells at no less than 0.4 x 10^6 cells/ml during that stage. After ~two passages, the cell growth rate increases, and the cells can be split to 0.2 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 cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~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

Assay conditions described have been optimized for these specific antibodies and cell lines. When testing other antibodies or target cells, different assay conditions (assay time, cell numbers, and target: effector cells ratio) may be required for optimum results. This protocol is a general guideline.

- The following assays are designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.
- The assay should be performed in triplicate.
- The assay should include Anti-HER2 antibody (if using HER2-expressing target cells), a test antibody (if applicable) and a non-specific control antibody.
- The assay should include cell-free wells to measure background luciferase activity as controls.
- The assay can include NFAT Reporter (Luc) – Jurkat Recombinant Cell Line as negative control.

Assay Medium: Thaw Medium 2.

A. Activation of ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat Cell Line in response to anti-HER2 IgG1 antibody, when co-cultured with BT-474 cells.

1. Seed 6 x 10^4 BT-474 cells into each well of a white clear-bottom 96-well microplate. Leave a couple of wells empty for use as the cell-free control.

2. Incubate cells at 37°C in a CO_2 incubator for 16-24 hours.

3. Prepare a solution of anti-HER2 antibody, Test Antibody, and non-specific control antibody at the desired concentrations in Assay Medium (50 µl/well).

4. Remove the media from the BT-474 cells.

5. Add 50 µl of diluted antibody to BT-474 cells.

6. Resuspend ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat cells at 1.2 x 10^6 cells/ml in Assay Medium (50 µl/well).

7. Add 50 µl of diluted ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat cells to the BT-474 antibody-treated cells.

8. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).

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

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

11. Rock gently at Room Temperature for ~15-30 minutes.

12. Measure luminescence using a luminometer.

13. 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 well divided by the average background-subtracted luminescence of non-specific antibody control wells.

$$Fold\ induction = \frac{average\ Lum\ sample - average\ backgound}{average\ Lum\ control - average\ background}$$

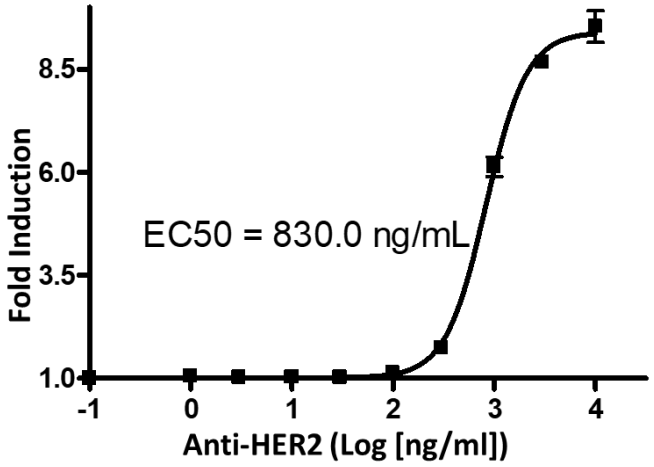

Figure 2: Dose-dependent response of ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with BT-474 cells in the presence of a humanized anti-HER2 IgG1 antibody.
ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat cells were incubated with increasing concentrations of anti-HER2 antibody, with or without the BT-474 target cells. NFAT activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold-induction (in which non-specific control is set to 1).

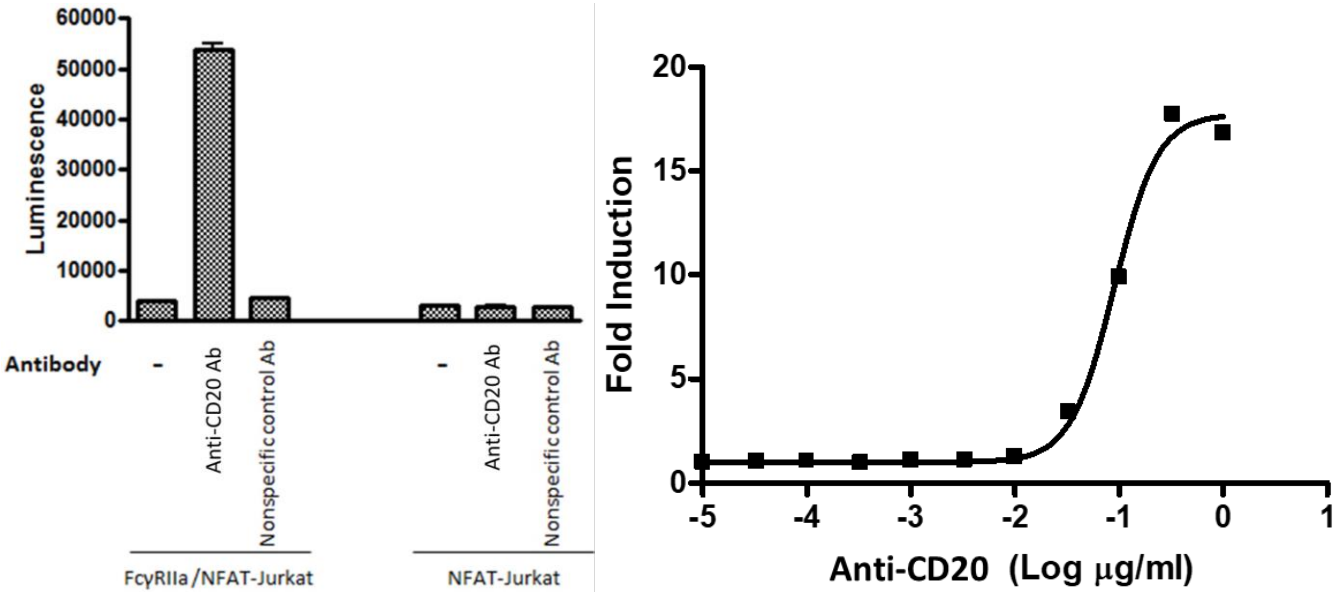

Figure 3: Activation of ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with Raji cells in the presence of anti-CD20 antibody.
Left panel: ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat cells respond to anti-CD20 antibody when co-cultured with CD20-expressing Raji cells, whereas NFAT Reporter (Luc) – Jurkat Cells, which do not express FcγRIIa (H Variant), were not activated. Results are expressed as raw luminescence signal. Right panel: ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat cells were incubated with increasing concentrations of anti-CD20 antibody, with or without Raji target cells. In both experiments, NFAT-dependent luciferase activity was measured with ONE-Step™ Luciferase Assay System.

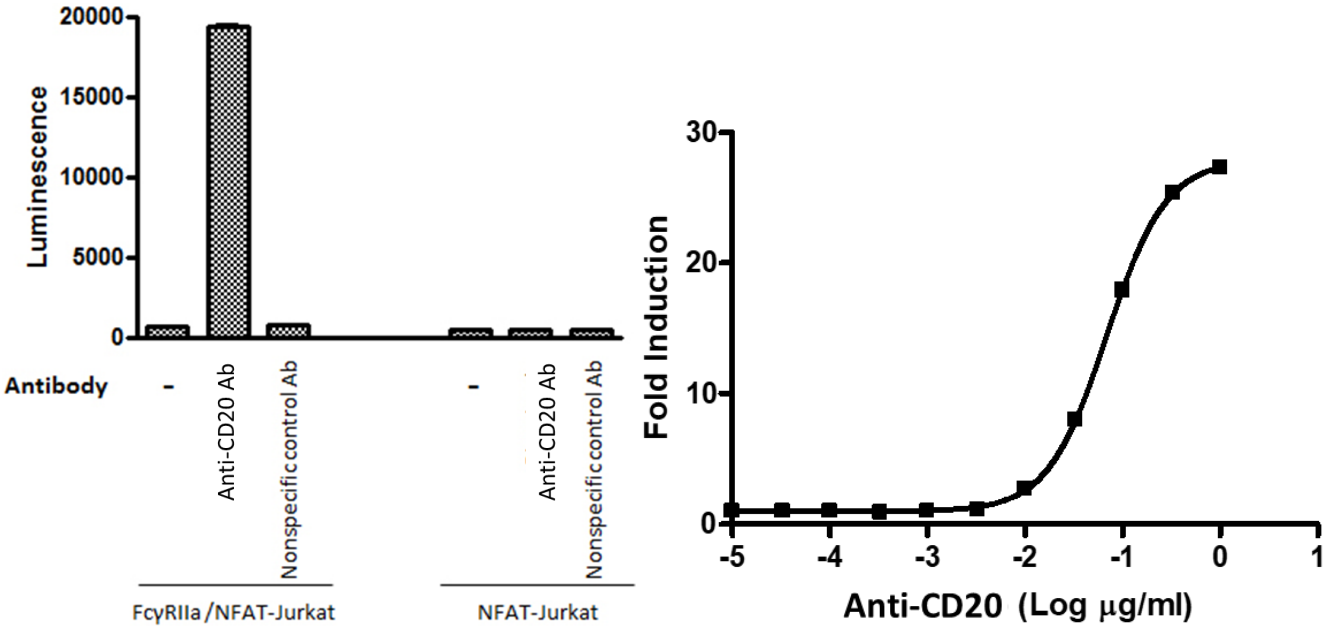

Figure 4: ADCP response of ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat Cell Line towards WIL2-S cells in the presence of anti-CD20 antibody.
Left panel: ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Jurkat cells were activated by anti-CD20 in the presence of CD20-expressing WIL2-S cells, whereas NFAT Reporter (Luc) – Jurkat Cells, which do not express FcγRIIa (H Variant), were not activated. Results are expressed as raw luminescence signal. Right panel: dose-dependent response of ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Luciferase Reporter Jurkat Cells co-cultured with WIL2-S cells in the presence of increasing concentrations of anti-CD20 antibody. In both experiments, NFAT-dependent luciferase activity was measured with ONE-Step™ Luciferase Assay System.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Cao X., *et al.*, 2022. *Science Advances* 8 (11): DOI: 10.1126/sciadv.abl9171

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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*
ADCC Bioassay Effector Cell F Variant (Low Affinity) Jurkat Cell Line	60540	2 vials
ADCC Bioassay Effector Cell V Variant (High Affinity) Jurkat Cell Line	60541	2 vials
ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line	79733	2 vials