Description

ADCC Bioassay Effector Cell F Variant (Low Affinity)/ NFAT Luciferase Reporter Jurkat Cell Line is an engineered Jurkat T cell line expressing firefly luciferase under the control of NFAT response elements, and human FcγRIIIa low affinity (F158) variant and Fcγ chain. This cell line was functionally validated by ADCC (antibody-dependent cell-mediated cytotoxicity) assays.

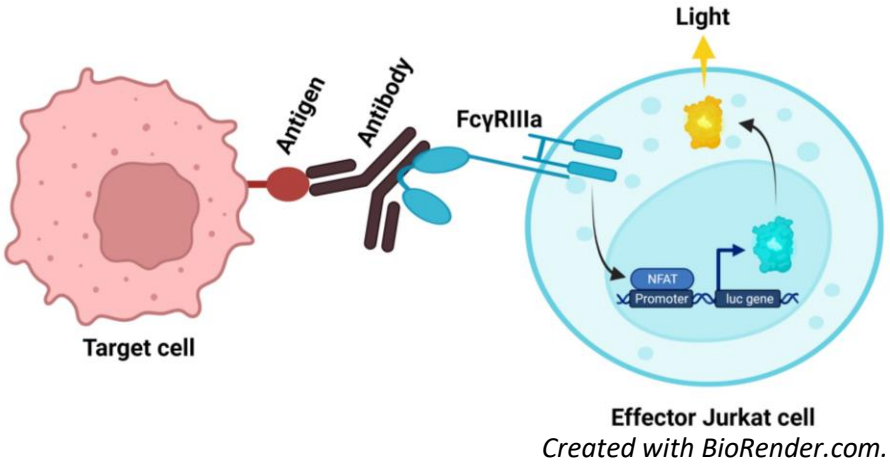

Figure 1: Illustration of the mechanism of action of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat Cell Line.

ADCC Bioassay Effector Cell F Variant (Low Affinity)/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 FcγRIIIa on the cell surface of the effector cell, cross-linking the effector and target cells. Engagement of FcγRIIIa leads to the activation of the NFAT pathway in the effector cells. Luciferase activity is proportional to the activation of the ADCC cascade.

Background

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune defense mechanism that involves an effector cell lysing a target cell on which antibodies have bound to specific antigens. The typical ADCC involves activation of natural killer (NK) cells by engaged antibodies. NK cells express Fc receptors, mostly FcγRIIIa (CD16a), on their surface. These Fc receptors recognize and bind the Fc portion of an antibody, such as IgG, when the IgG is bound to the surface of a pathogen-infected target cell. Once the Fc receptor binds to the Fc region of IgG, the NK cell releases cytokines such as IFN-γ and cytotoxic molecules that attack the pathogen-infected target cell. Human FcγRIIIa displays a dimorphism at residue 158. F FcγRIIIa V158 encodes a higher affinity receptor variant with a valine at amino acid residue 158, while F158 encodes a lower affinity receptor variant with a phenylalanine at amino acid residue 158.

Application

- Screen and optimize Fc effector function of antibodies in ADCC assays.
- Measure ADCC 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)

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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
SK-BR-3 Cells	ATCC #HTB-30
Human B Cells WIL2-S	ATCC #CRL-8885
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.

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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:
Assay Medium 2A (BPS Bioscience #79621):
RPMI 1640 medium supplemented with 10% low IgG FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol
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 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 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 0.1 x 10^6 cells/ml. We recommend a sub-cultivation ratio of 1:10 to 1:20 twice a week.

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.

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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) or anti-CD20 antibody (if using CD20-expressing target cells) as positive controls, a test antibody (if applicable) and a non-specific control antibody.
- The assay should include cell-free wells to measure background luciferase activity.

Assay Medium: Assay Medium 2A.

A. ADCC activity of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat Cell Line in response to anti-HER2 IgG1 antibody, when co-cultured with SK-BR-3 cells.

1. Grow ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells in Assay Medium.

2. Incubate cells at 37°C in a CO_2 incubator for 24-48 hours.

3. Prepare a suspension of SK-BR-3 cells at 1.2 x 10^5/ml in SK-BR-3 media (such as McCoy's 5A + 10% FBS + 1% Penicillin/Streptomycin).

4. Plate 100 µl of SK-BR-3 cell suspension into each well of a white clear-bottom 96-well microplate. Leave a few wells empty (no cells) for the background luminescence control.

5. Incubate cells at 37°C in a CO_2 incubator for 24 hours.

6. Prepare a 1 µg/ml solution of positive control anti-HER2 antibody (60 µl/well).

7. Prepare the Test antibody and the non-specific control antibody at the desired concentrations in Assay Medium (60 µl/well).

8. Remove the medium from the wells of SK-BR-3 cells.

9. Add 60 µl of diluted controls and Test antibodies to SK-BR-3 cells.

10. Incubate the plate at 37°C in a CO_2 incubator for 1 hour.

11. Resuspend ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells at 1.8 x 10^6 cells/ml in Assay Medium (40 µl/well).

12. Add 40 µl of diluted ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells to the wells with SK-BR-3 antibody-treated cells.

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

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

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

16. Rock gently at Room Temperature (RT) for ~30 minutes.

17. Measure luminescence using a luminometer.

18. 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.

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

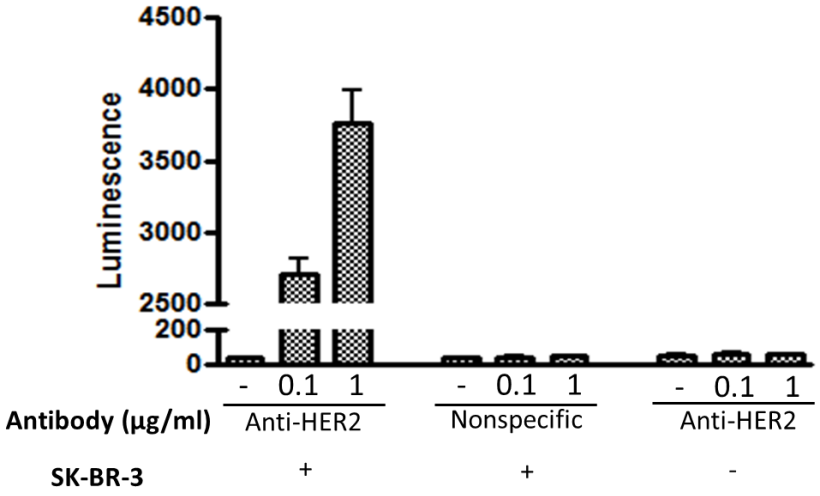

Figure 2: ADCC response of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with SK-BR-3 cells in the presence of an anti-HER2 humanized IgG1 antibody.
ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells were incubated with anti-HER2 or non-specific antibody, with or without SK-BR-3 target cells. NFAT activity was measured with ONE-Step™ Luciferase Assay System.

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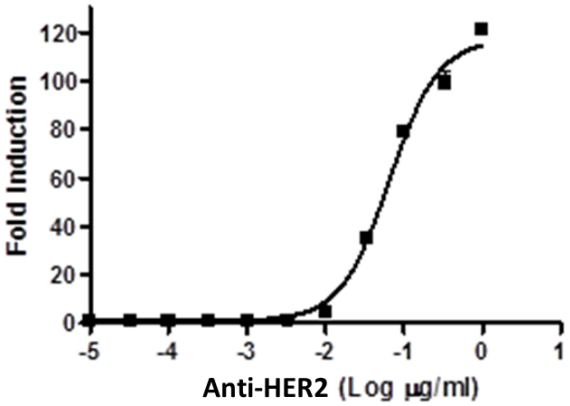

Figure 3: ADCC response of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with SK-BR-3 in the presence of anti-HER2 antibody.
SK-BR-3 cells were incubated with increasing concentrations of anti-HER2 antibody and co-cultured with ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells. NFAT activity was measured with ONE-Step™ Luciferase Assay System. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of non-specific control.

B. ADCC activity of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat Cell Line in response to anti-CD20 antibody, when co-cultured with WIL2-S cells.

1. Grow ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells in Assay Medium.
2. Incubate cells at 37°C in a CO_2 incubator for 24-48 hours.
3. Prepare a 2 µg/ml solution of anti-CD20 antibody (30 µl/well).
4. Prepare the Test antibody and the negative control antibody at 2x the desired concentrations in Assay Medium (30 µl/well).
5. Dilute WIL2-S cells at 4 x 10^5/ml in Assay Medium.
6. Plate 30 µl of WIL2-S cell suspension into each well of a white clear-bottom 96-well microplate. Leave a few wells empty (no cells) for the background luminescence control.
7. Add 30 µl of antibody solution to the WIL2-S containing wells.
8. Incubate the plate at 37°C in a CO_2 incubator for 1 hour.
9. Resuspend ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells at 1.8 x 10^6 cells/ml in Assay Medium (40 µl/well).
10. Add 40 µl of diluted ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells to the wells with WIL2-S antibody-treated cells.
11. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).

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

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

14. Rock gently at Room Temperature (RT) for ~30 minutes.

15. Measure luminescence using a luminometer.

16. 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 untreated control wells.

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

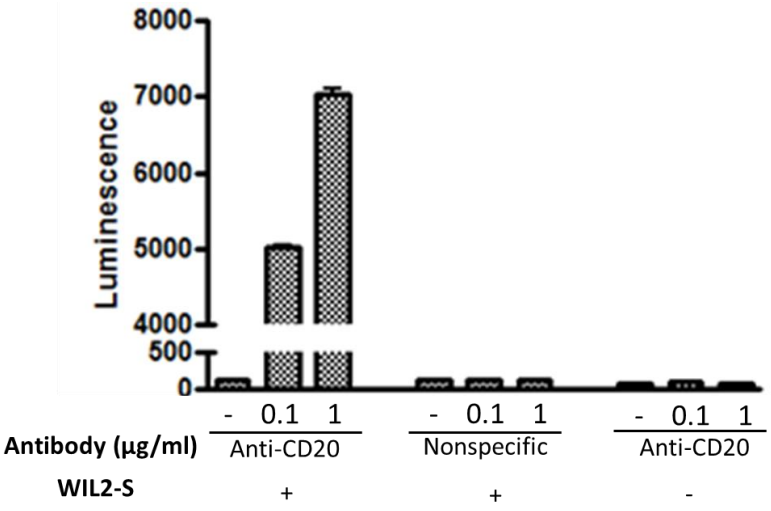

Figure 4: ADCC response of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with WIL2-S cells in the presence of an anti-CD20 antibody. ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells were incubated with anti-CD20 and nonspecific antibody, with or without WIL2-S target cells. NFAT activity was measured with ONE-Step™ Luciferase Assay System.

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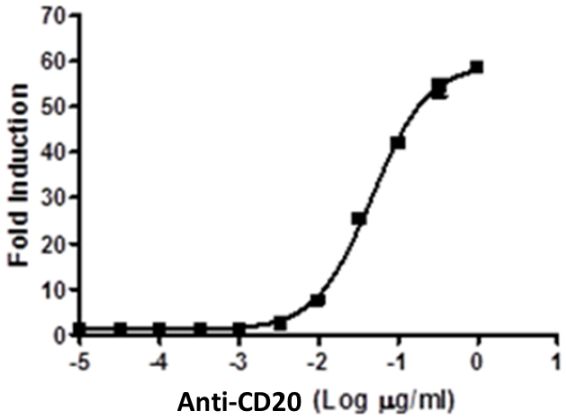

Figure 5: ADCC response of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with WIL2-S in the presence of anti-CD20 antibody.
WIL2-S cells were incubated with increasing concentrations of anti-CD20 antibody in the presence of ADCC Bioassay Effector Cell F Variant (Low Affinity)/NFAT Luciferase Reporter Jurkat cells. NFAT activity was measured with ONE-Step™ Luciferase Assay System. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control.

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 V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat Cell Line	60541	2 vials
ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line	79733	2 vials
ADCP Bioassay Effector Cell FcγRIIa (H Variant)/NFAT Reporter Jurkat Cell Line	71273	2 vials

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