

Description

ADCP Bioassay Effector Cell FcγRI (CD64)/NFAT Reporter Jurkat Cell Line is a Jurkat T cell line engineered to express firefly luciferase under the control of NFAT (nuclear factor of activated T cells) response elements, and human FcγRI (Fc-gamma receptor 1), also known as CD64.

This cell line was functionally validated in a co-culture assay using antigen-expressing cells in the presence of a relevant antibody.

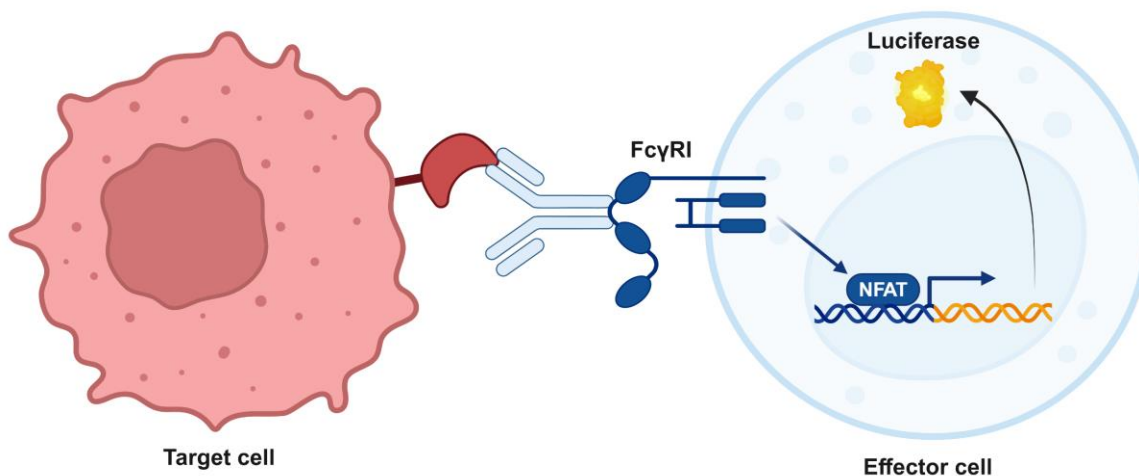


Figure 1: Illustration of the mechanism of action of ADCP Bioassay Effector Cell FcγRI (CD64)/NFAT Luciferase Reporter Jurkat Cell Line.

ADCP Bioassay Effector Cell FcγRI (CD64)/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 FcγRI on the cell surface of the effector cell, cross-linking the effector and target cells. Engagement of FcγRI 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 to consider during antibody drug development. FcγRI (Fc gamma receptor 1), also known as CD64, is found in macrophages and monocytes, and its expression can be induced in polymorphonuclear leukocytes by IFNγ (interferon gamma) and G-CSF (granulocyte colony-stimulating factor). Multivalent IgG immune complexes (ICs) promote receptor clustering and activation of intracellular signaling through phosphorylation of ITAMs (immunoreceptor tyrosine-based activation motifs), 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 (CLL). However, an overactivation of FcγRI can result in chronic inflammation and autoimmune disorders, such as SLE (systemic lupus erythematosus), ITP (immune thrombocytopenia) and RA (rheumatoid arthritis). Leveraging FcγRI properties can be beneficial in cancer therapy, while blocking its activation is a promising therapeutic approach for autoimmune disorders.

Application

- Screen and validate the 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 (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 2A	BPS Bioscience #60190

Materials Required for Cellular Assays

Name	Ordering Information
Anti-CD20 Functional Antibody (Rituximab)	BPS Bioscience #71209
Raji Cells	ATCC #CCL-86
NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60621
96-well white, clear-bottom cell culture 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% CO₂. 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 (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin, and 200 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium:

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium (ATCC modification) 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% CO₂ 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% CO₂ incubator at 37°C until the cells are ready to passage.
7. Cells should be passaged before they reach 2.5 x 10⁶ 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 0.2×10^6 cells/ml. We recommend a sub-cultivation ratio of 1:10 twice a week to maintain the cell density between 0.2×10^6 cells/ml to 2.5×10^6 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 0.4×10^6 cells/ml during that stage. After two to three passages, the cell growth rate increases, and the cells can be split to 0.2×10^6 cells/ml.

Cell Freezing

1. Spin down the cells at $300 \times g$ for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at $\sim 2 \times 10^6$ 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

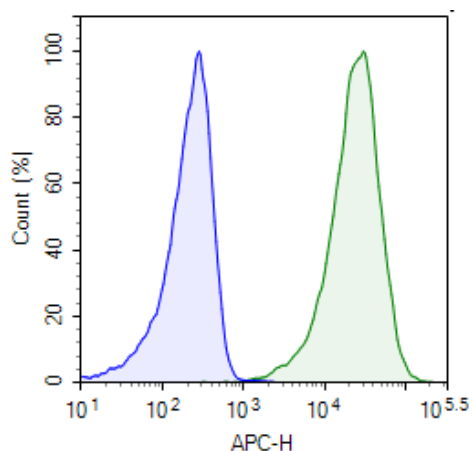


Figure 2. Cell surface expression of FcγRI in ADCP Bioassay Effector Cell FcγRI (CD64) NFAT Luciferase Reporter Jurkat Cell Line by flow cytometry.

ADCP Bioassay Effector Cell FcγRI (CD64) NFAT Luciferase Reporter Jurkat cells (green) and control NFAT Luciferase Reporter Jurkat cells (blue) were stained with an APC-conjugated CD64 antibody and analyzed by flow cytometry. Y-axis represents the cell count. X-axis indicates APC intensity.

Functional Validation

- The 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 only.
- 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 a “Cell-Free Control”, “Untreated Control” and “Treated” Conditions.
- The use of a validated antibody, such as anti-CD20 functional antibody (Rituximab) if using CD20-expressing target cells, and a non-specific control antibody are recommended.
- The assay can include NFAT Luciferase Reporter Jurkat Cell Line as negative control.

Assay Medium: Thaw Medium 2.

A. Activation of ADCP Bioassay Effector Cell FcγRI (CD64)/NFAT Luciferase Reporter Jurkat Cell Line in response to an anti-CD20 IgG1 antibody (Rituximab), when co-cultured with Raji cells.

1. Resuspend Raji cells in log phase growth at 6×10^5 cells/ml in Assay Medium.
2. Add 50 μ l of Raji cell suspension to each well of a 96-well white clear-bottom cell culture plate. Leave a couple of wells empty for use as the “Cell-Free Control”.
3. Prepare a solution of anti-CD20 antibody at a concentration 4x higher than the desired final concentration in Assay Medium (25 μ l/well).
4. Add 25 μ l of diluted anti-CD20 antibody to the “Treated” Raji cell wells.
5. Add 25 μ l of Assay Medium to the “Untreated Control” Raji cell wells.
6. Incubate cells with the antibodies at 37°C in a CO₂ incubator for 30 minutes.
7. Harvest and resuspend ADCP Bioassay Effector Cell FcγRI (CD64)/NFAT Luciferase Reporter Jurkat cells at 1.2×10^6 cells/ml in Assay Medium (25 μ l/well).
8. Add 25 μ l of ADCP Bioassay Effector Cell FcγRI (CD64)/NFAT Luciferase Reporter Jurkat cells to the “Treated” and “Untreated Control” Raji cell wells.
9. Add 100 μ l of Assay Medium to the “Cell-Free Control” wells (for determining background luminescence).
10. Incubate the plate at 37°C in a CO₂ incubator for 5 hours.
11. Add 100 μ l of the ONE-Step™ Luciferase reagent per well.
12. Rock gently at Room Temperature (RT) for ~15-30 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. The fold induction of NFAT luciferase reporter expression is the background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of untreated control wells.

$$\text{Fold induction} = \frac{\text{average Lum treated wells} - \text{average background}}{\text{average Lum untreated wells} - \text{average background}}$$

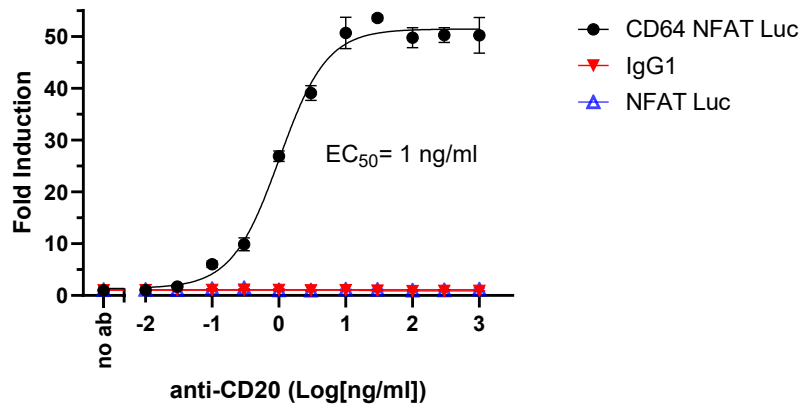


Figure 3: Activation of ADCP Bioassay Effector Cell FcγRI (CD64)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with Raji cells in the presence of Anti-CD20 Functional Antibody (Rituximab). ADCP Bioassay Effector Cell FcγRI (CD64) NFAT Luciferase Reporter Jurkat cells (effector) were co-cultured with Raji cells as described in the protocol above in the presence of Anti-CD20 Functional Antibody (Rituximab). Cross-linking of the anti-CD20 antibody by FcγRI expressed at the surface of the effector cells allowed their activation (Black). When the effector cells were co-cultured with Raji cells in the presence of human IgG1 isotype control, no activation of NFAT was observed (Red). When control NFAT Luciferase Reporter Jurkat cells were co-cultured with Raji cells in the presence of the anti-CD20 antibody, no activation of the control cells was observed (Blue).

Data shown is representative.

References

Holtrop T., *et al.*, 2025 *Nature Communications* 16 :10068.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

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