Description

The ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line (mFcγRIV /NFAT-Jurkat cells) are engineered Jurkat cells that express firefly luciferase, under the control of NFAT response elements, and mouse FcγRIV (NM_144559.2). This cell line was functionally validated in an ADCC (antibody-dependent cell-mediated cytotoxicity) assay.

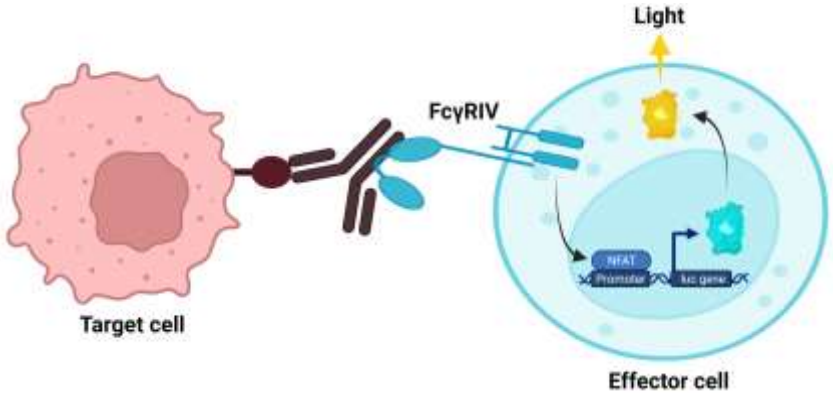

Figure 1: Illustration of the mechanism of action of ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line. ADCC Bioassay Effector Cell (Mouse) Jurkat cells are used as effector cells. The Fc effector portion of antibodies binds to target antigens on the target cell surface and also binds to mFcγRIV on the cell surface of effector cells, cross-linking of the effectors and target cells. Binding to mFcγRIV leads to the activation of NFAT pathway in the effector cells. Luciferase activity is proportional to the amount of crosslinking and activation of the ADCC cascade.

Background

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune defense mechanism involving an effector immune cell lysing a target cell on which antibodies have bound to specific antigens. The typical ADCC involves activation of natural killer (NK) cells by antibodies engaged on a target. NK cells express Fc receptors, mostly FcγRIV in mouse, on their cell surface. These Fc receptors recognize and bind to the Fc portion of antibodies, usually IgG2a, IgG2b and IgG3 in mouse, present on 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.

Application

- Screen and validate 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)

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 2G	BPS Bioscience #79734

Materials Required for Cellular Assays

Name	Ordering Information
Assay Medium 2A	BPS Bioscience #79621
Anti-CD20 Mouse IgG2a	InvivoGen #hcd20-mab10
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.

Growth Medium 2G (BPS Bioscience #79734):

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 mg/ml G418 and 2.5 µg/ml Puromycin.

Assay Medium:

Assay Medium 2A: RPMI 1640 medium supplemented with 10% low IgG 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 Thaw Medium, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. 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 2G.

Cell Passage

Dilute cell suspension into new culture vessels with Growth Medium 2G at no less than 0.1 x 10^6 cells/ml. We recommend a subcultivation ratio of 1:10 to 1:20 twice a week.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~1:4 ratio at the beginning of the culture. After several passages, the cell growth rate increases and the cells can be split at higher ratio.

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 anti-CD20 IgG2a and WIL2-S target cells. 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-CD20 mouse, a test antibody (if applicable) and a control antibody.
- The assay should include cell-free wells and untreated wells as controls.

Assay Medium: Assay Medium 2A.

A. ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line activity in response to mouse anti-CD20 IgG2 antibody towards WIL2-S cells.

1. Seed ADCC Bioassay Effector Cell (Mouse) Jurkat cells into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 2. 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 24-48 hours.
3. Seed WIL2-S cells into a white opaque-bottom 96-well microplate at 1 x 10^4 cells/well in Assay Medium.
4. Prepare a solution of anti-CD20, test and control antibody at the appropriate concentration in Assay Medium.
5. Add anti-CD20 mouse antibody, test antibody or control antibody to the wells with WIL2-S cells.
6. Mix and incubate cells at 37°C in a CO_2 incubator for 1 hour.
7. Ressuspend ADCC Bioassay Effector Cell (Mouse) Jurkat cells at 6 x 10^5 cells/ml in Assay Medium 2A.
8. Add 100 µl of diluted ADCC Bioassay Effector Cell (Mouse) Jurkat cells to the wells with WIL2-S antibody-treated cells.
9. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).
10. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.
11. Add 100 µl of the ONE-Step™ Luciferase reagent per well.
12. Rock gently at Room Temperature (RT) for ~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 = background-subtracted luminescence of treated well / average background-subtracted luminescence of untreated control wells.

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

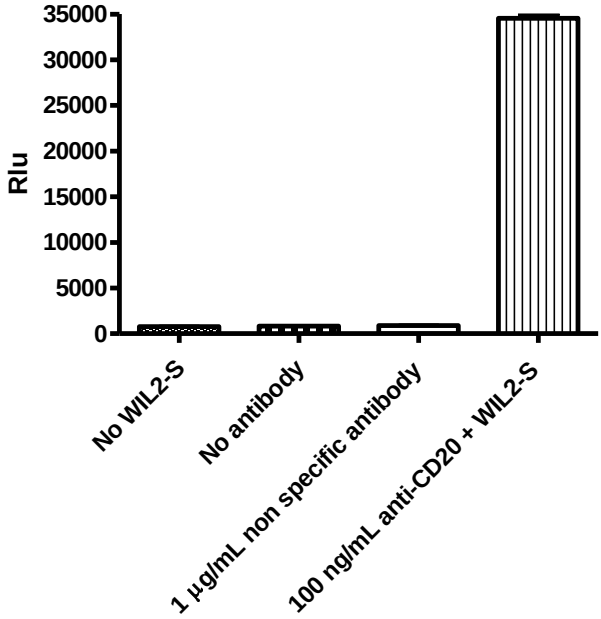

Figure 2: Response of ADCC Bioassay Effector Cell (Mouse) -Jurkat Recombinant Cell Line towards WIL2-S cells when in the presence of anti-CD20 mouse IgG2a antibody.
Anti-CD20, nonspecific control antibody, or Assay Medium (no antibody) were incubated with ADCC Bioassay Effector Cell (Mouse) Jurkat cells, with or without the WIL2-S target cells. NFAT activity was measured with ONE-Step™ Luciferase Assay System.

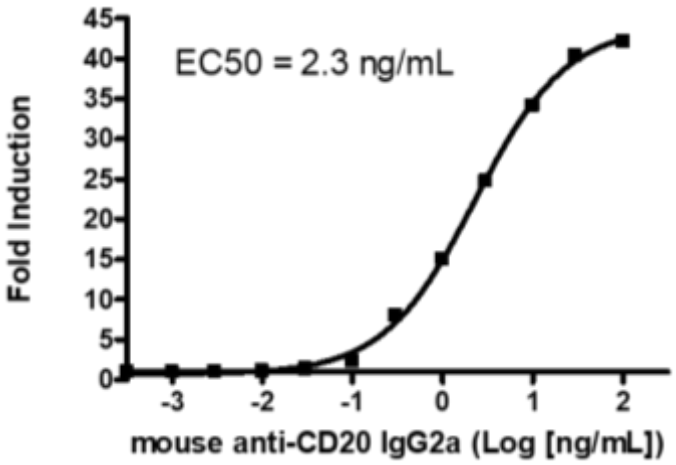

Figure 3: Dose-dependent response of ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line to anti-CD20 mouse IgG2a antibody.
ADCC Bioassay Effector Cell (Mouse) Jurkat cells, were incubated with increasing concentrations of anti-CD20 mouse antibody. NFAT activity was measured with ONE-Step™ Luciferase 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

Parekh B., *et al.* 2012. *mAbs* 4(3):310-318, doi:10.4161/mabs.19873
Rosales C., 2017. *Frontiers in Immunology* 8: 280, doi:10.3389/fimmu.2017.00280

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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) Jurkat Cell Line	60541	2 vials
ADCC Bioassay Effector Cell V Variant (High Affinity) Jurkat Cell Line	60540	2 vials
Anti-CD20-Anti-CD3 Bispecific Antibody	100836	50 µg/100 µg
Anti-CD20 Functional Antibody	71209	100 µg