6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

ADCC Bioassay Effector Cell
V variant (High Affinity)
Catalog #: 60541

Product Description
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of human FcγRIIIa, high affinity (V158) variant and Fcγ chain.

Background
Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune defense mechanism involving an effector cell lysing a target cell on which antibodies have bound to specific antigens on the target cell membrane. The typical ADCC involves activation of natural killer (NK) cells by antibodies. NK cells express Fc receptors, mostly CD16 or FcγRIIIa (CD16a), on its cell surface. These Fc receptors recognize and bind to the Fc portion of an antibody, such as IgG, which has bound to the surface of a pathogen-infected target cell. Once the Fc receptor binds to the Fc region of IgG, the Natural Killer cell releases cytokines such as IFN-γ and cytotoxic molecules that attack the pathogen-infected target cell.
The human FcγRIIIa displays a dimorphism in the position of residue 158. One allele (V158) encodes a higher Fc affinity receptor variant with a valine at amino acid residue 158, and the other (F158) encodes a lower Fc affinity receptor variant having a phenylalanine at amino acid residue 158.

Application
Characterize the Fc effector function of antibodies and measure ADCC activity in cellular assays.

Format
Each vial contains ~2 x 10^6 cells in 1 ml of 10% DMSO.

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

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General Culture Conditions

Thaw Medium 2 (BPS Cat. #60184): RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2A (BPS Cat. #60190): Thaw Medium 2 (BPS Cat. #60184) plus 1 mg/ml of Geneticin (Life Technologies #11811031) and 200 µg/ml of Hygromycin B (Hyclone #SV30070.01).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2A.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. This cell line tends to grow more slowly than parental Jurkat cells. After 24 hours of culture, add an additional 3 – 4 ml of Thaw Medium 2 **(no Geneticin and Hygromycin B)**. At first passage, switch to Growth Medium 2A **(contains Geneticin and Hygromycin B).** Cells should be split before they reach 2 x 10^6 cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.1 x 10^6 cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

Functional Validation

The functionality of the cell line was validated using an ADCC reporter assay. In this ADCC assay, the engineered Jurkat cells stably expressing human FcγRIIIa, and firefly luciferase gene under the control of NFAT response elements (FcγRIIIa /NFAT-Jurkat cells) are used as effector cells. An antibody is bound to the target antigen on the target cell surface. When the Fc portion of the antibody also binds to FcγRIIIa on the surface of effector cells, cross-linking of the effector and target cells occurs. This cross-linking leads to activation of the NFAT pathway and luciferase expression.

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Protocol

Sample protocol for testing SK-BR-3 cells with Trastuzumab (Herceptin, Anti-HER2)

1. One or two days before assay, seed ADCC cells in Assay Medium 2A (#79621) and grow for 1 or 2 days.

   Assay Medium 2A (BPS Cat. #79621): RPMI + low IgG FBS 10% + 1% Penicillin/Streptomycin

   One day before assay, seed SK-BR-3 cells (or other target cells) at a density of 12,000 cells per well into white clear-bottom 96-well microplate in 100 µl of Assay Medium 2A. Leave a few wells empty (no cells) for the background luminescence control.
2. On the day of the assay, remove medium from the plate, add in antibody with 60 µl Assay Medium 2A (1 µg/mL Trastuzumab or nonspecific negative control antibody). Mix well and incubate cells with antibody at 37°C in a CO2 incubator for 1 hour.
3. Harvest the ADCC/NFAT-reporter-Jurkat cells by centrifugation and resuspend in Assay Medium 2A at 75,000 ADCC cells/40 µl. Add to the 60 µl of SK-BR-3 cells incubated with either Trastuzumab or nonspecific negative control antibody. Set up each treatment in at least triplicate.
4. Add 100 µl of Assay Medium 2A to cell-free control wells (for determining background luminescence). Incubate the plates at 37° in a CO2 incubator for 5 to 6 hours.
5. After ~5 to 6 hour incubation, perform luciferase assay using the ONE-Step luciferase assay system (#60690) following the provided protocol. Add 100 µl of One-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.
6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Sample protocol for testing Wil-2S cells with Rituxan

1. One or two days before assay, seed ADCC cells in Assay Medium 2A and grow for 1 or 2 days.
2. On the day of assay, harvest Wil-2S cells from culture and seed cells at a density of 12,500 cells per well into white clear-bottom 96-well microplate in 60 µl of Assay Medium 2A containing either the testing antibody (1 µg/mL Rituxan) or the nonspecific negative control antibody. Mix well and incubate cells with antibody at 37°C in a CO2 incubator for 1 hour.
3. Harvest the ADCC/NFAT-reporter-Jurkat cells by centrifugation and resuspend at 75000 ADCC cells in 40 µl Assay Medium 2A. Add the 40 µl of ADCC cells to the 60 ul of Wil-2S cells. Set up each treatment in at least triplicate.

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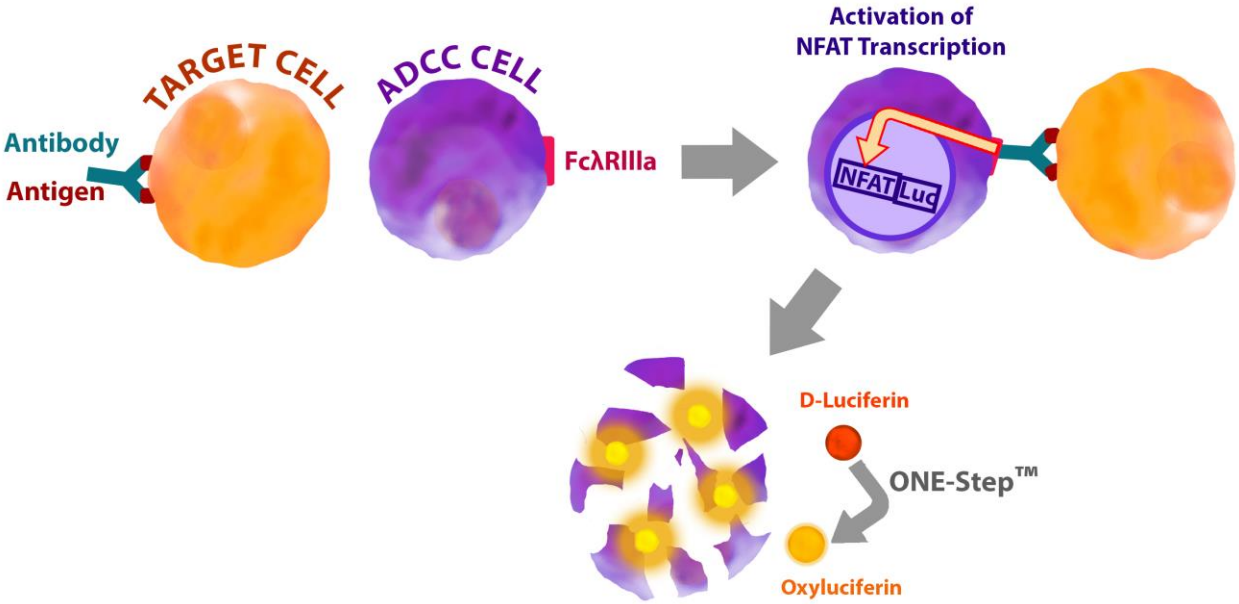
4. Add 100 µl of Assay Medium 2A to cell-free control wells (for determining background luminescence). Incubate the plates at 37° in a CO2 incubator for 5 to 6 hours.
5. After ~5 to 6 hour incubation, perform luciferase assay using the ONE-Step luciferase assay system (#60690) following the provided protocol. Add 100 µl of One-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.
6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Assay Principle



Materials Required But Not Supplied for Cell Culture

- Thaw Medium 2 (BPS Cat. #60184)
- Growth Medium 2A (BPS Cat. #60190)
- Geneticin (Life Technologies #11811031)
- Hygromycin B (Hyclone #SV30070.01)

Materials Required But Not Supplied for Cellular Assay

- Assay Medium 2A (BPS Cat. #79621)
- Anti-CD20, BPS #71209
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience # 60690)
- Luminometer

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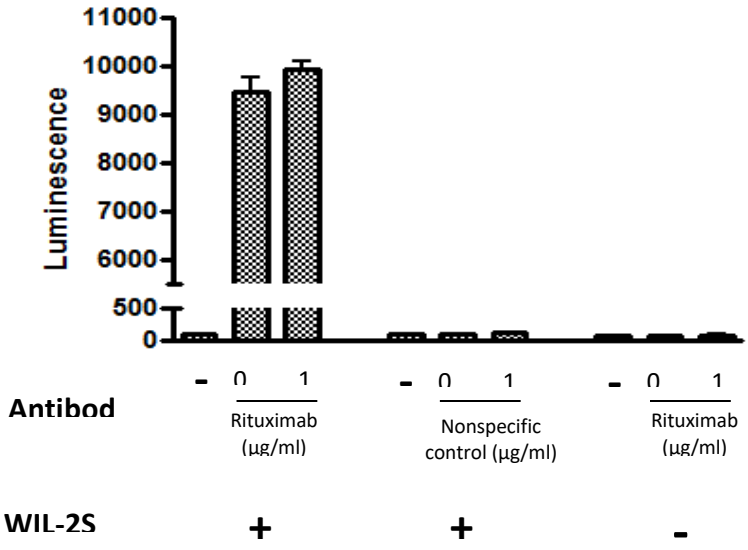
Figure 1. ADCC response to Rituximab, an anti-CD20 chimeric IgG1 antibody drug, in ADCC Bioassay Effector Cell (FcγRIIIa (V158) /NFAT-Jurkat cells).
Rituximab (Anti-CD20, BPS #71209), nonspecific control antibody, or Assay Medium 2A (no antibody) was incubated with ADCC Bioassay Effector Cells (FcγRIIIa (V158)/NFAT-Jurkat cells), with or without target cells (human B cell WIL2-S). After ~5 hours of stimulation, ONE-Step™ Luciferase reagent (BPS Cat. #60690) was added to the cells to measure NFAT activity.

A. Specificity of the ADCC response to Rituximab. Rituximab induced NFAT luciferase reporter activity in ADCC Bioassay Effector Cell (FcγRIIIa (V158)/NFAT-Jurkat cells) co-cultured with WIL2-S.



B. Dose response of Rituximab in ADCC Bioassay Effector Cell (FcγRIIIa (V158)/NFAT-Jurkat cells). The result is shown as fold induction of NFAT luciferase reporter. EC50 = 13.4 ng/ml.

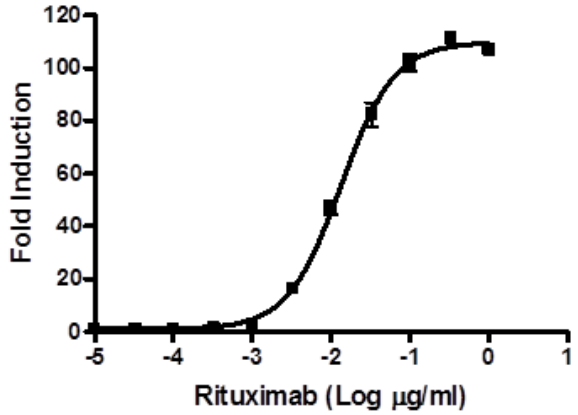


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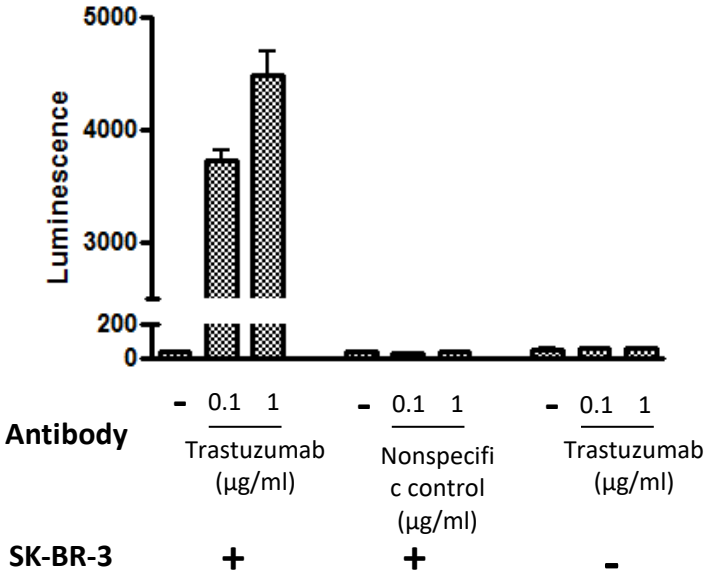
Figure 2. ADCC response to Trastuzumab, an anti-HER2 humanized IgG1 antibody drug, in ADCC Bioassay Effector Cell (FcγRIIIa (V158)/NFAT-Jurkat cells).
Trastuzumab (anti-HER2), nonspecific control antibody, or Assay Medium 2A (no antibody) was incubated with ADCC Bioassay Effector Cell (FcγRIIIa (V158)/NFAT-Jurkat cells), with or without target cells (human breast cancer cell SK-BR-3). After ~5 hours of stimulation, ONE-Step™ Luciferase reagent (BPS Cat. #60690) was added to the cells to measure NFAT activity.

A. Specificity of the ADCC response to Trastuzumab. Trastuzumab induced NFAT luciferase reporter activity in ADCC Bioassay Effector Cell (FcγRIIIa (V158)/NFAT-Jurkat cells) co-cultured with SK-BR-3.



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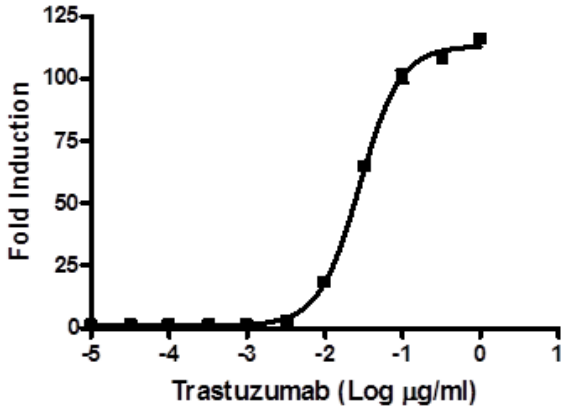
B. Dose response of Trastuzumab in ADCC Bioassay Effector Cell (FcγRIIIa (V158)/NFAT-Jurkat cells). The result is shown as fold induction of NFAT luciferase reporter. EC50 = 28.1 ng/ml



References

1. Koene, HR, *et al. Blood*. 1997; **90:**1109–14.
2. Wu, J, *et al. J Clin Invest*. 1997; **100:**1059–70.

Related Products

Product	Cat. #	Size
ADCC Bioassay Effector Cell, F variant (Low Affinity)	60540	2 vials
NFAT Reporter – Jurkat cell line	60621	2 vials
Anti-CD20 antibody	71209	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 2	60184	100 ml

Notes

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees**;** contact *sales@bpsbioscience.com* for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.