SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

ADCP Bioassay Effector Cell
FcγRIIa (H variant) /NFAT Reporter-Jurkat
Catalog #: 71273

Product Description
Recombinant Jurkat T cell expressing a firefly luciferase gene under the control of NFAT response elements with constitutive expression of human FcγRIIa, Histidine variant.

Background
Antibody-dependent cell-mediated phagocytosis (ADCP) is one of the important mechanisms of action for antibody drug development. FcγRIIa 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 the activation of these effector cells. Several clinical studies have studied the correlation of a FcγRIIa polymorphism (R131H) and the response to IgG1 subclass monoclonal antibodies (mAbs) such as rituximab. Engineered amino-acid substitutions in Fc-mAbs have been developed to enhance the mAb-mediated phagocytosis of tumor cells by macrophages.

Application
- Characterize the Fc effector function of antibodies.

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, cat. #MP0025) to confirm the absence of *Mycoplasma* species.

Culture Medium and Recommended 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) + 1 mg/ml of Geneticin (Life Technologies, #11811031) and 200 µg/ml of Hygromycin B (Hyclone, #SV30070.01).

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Cells should be grown at 37°C with 5% CO_2 using **Growth Medium 2A** (Thaw Medium 2 + 1 mg/ml of Geneticin + 200 µg/ml of Hygromycin B).

If culturing cells in medium from other vendors, it may be required to adjust the percentage of CO_2 in the incubator, depending on the $NaHCO_3$ level in the basal medium

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 (**no Geneticin, no Hygromycin B)**, spin down cells at 1500 rpm, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 2 (**no Geneticin, no Hygromycin B)**. Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, add an additional ~3 ml of fresh warm Thaw Medium 2 (**no Geneticin, no Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach 2.5 x10^6 cells/ml. At first passage switch to Growth Medium 2A (**contains Geneticin and Hygromycin B)**.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml. Subcultivation ratio: ~1:10 twice a week, maintaining cell density at 0.2 x 10^6 cells/ml to 2.5 x10^6 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 x10^6 cells/ml *at the beginning of culturing. After ~two passages, the cell growth rate increases and the cells can be split to 0.2x10^6 cells/ml.*

To freeze down the cells, spin down cells, and resuspend cell pellet in 4°C Freezing Medium (10% DMSO + 90% FBS) to ~2x10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for long-term storage.
It is recommended to expand the cells and at early passage, freeze down 10 or more vials of cells for future use.

Functional Validation

The functionality of the cell line was validated using an ADCP reporter assay. In this ADCP assay, the effector cells are the FcγRIIa /NFAT Reporter-Jurkat cells, which are engineered Jurkat cells stably expressing human FcγRIIa, and firefly luciferase gene under the control of NFAT response elements. The Fc effector portion of antibodies that bind to target antigens on the target cell surface also binds to FcγRIIa on the cell surface of effector cells. This cross-linking of the effector and target cells leads to the activation of the NFAT pathway in the effector cells, an early step in ADCP MOA pathway, and the subsequent expression of luciferase.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

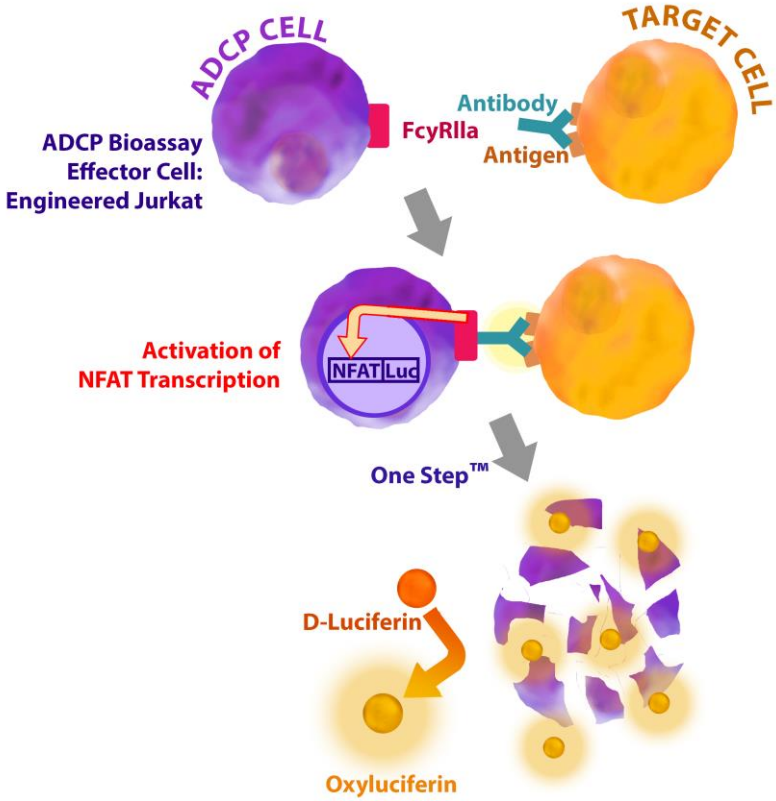


Figure 1. ADCP response to anti-CD20 (Rituximab), a chimeric IgG1 antibody drug, in ADCP Bioassay Effector Cell (FcγRIIa /NFAT Reporter-Jurkat cells), co-cultured with Raji cells.
Anti-CD20 (BPS, #71209), nonspecific control antibody, or assay medium (no antibody) was incubated with ADCP Bioassay Effector Cell (FcγRIIa /NFAT Reporter-Jurkat cells) or NFAT Reporter-Jurkat Cell (BPS, #60621), co-cultured with target cells (human B lymphocyte Raji cells). After ~5 hours of stimulation, ONE-Step™ Luciferase reagent (BPS, Cat. #60690) was added to the cells to measure NFAT activity.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Materials Required but Not Supplied

- **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).
- Anti-hHER2 human IgG1 (R&D # MAB9589-SP)
- BT-474 (ATCC #HTB-20)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

Analysis of human ADCP - Jurkat reporter activity in response to human anti-HER2 IgG1 antibody in co-culture with BT-474.

1. One day before assay, seed BT-474 cells in a white opaque 96-well plate, at 6 x 10^4 cells/well, let cells attach overnight at 37°C with 5% CO_2.

2. On the day of assay, remove culture medium from plate and add anti-HER2 human antibody IgG1 (R&D Cat# MAB9589-SP) or test antibody or control antibody in 50 µl Thaw Medium 2/well, incubate for one hour at 37°C with 5% CO_2.

3. Harvest the ADCP/NFAT-reporter-Jurkat cells by centrifugation and resuspend in Thaw Medium 2. Add 6 x 10^4 cells/well in 50 µl to the TB-474 cells incubated with either anti-HER2 or nonspecific negative control antibody. Set up each treatment in at least triplicate. Final assay volume is 100 µl/well.

4. Add 100 µl of Thaw Medium 2 to cell-free control wells (for determining background luminescence). Incubate the plates at 37°C in a CO_2 incubator for 5 hours.

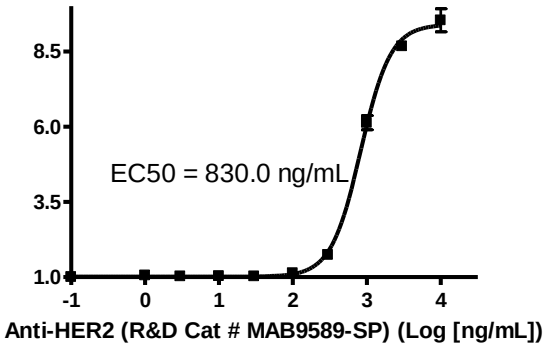
5. After ~5 hours of incubation, perform luciferase assay using the ONE-Step luciferase assay system (#60690) following the provided protocol. Add 100 µl of ONE-Step Luciferase reagent per well and rock gently at room temperature for ~15-30 minutes. Measure luminescence using a luminometer.

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Note: Assay conditions have been optimized for anti-HER2 IgG1 and BT-474 co-culture. When testing with other antibodies or target cells, different assay conditions may be required for optimum results, such as assay time, cell numbers, and target : effector cells ratio (1:1 in this assay protocol).

Figure 1. ADCP dose response of anti-HER2 human IgG1 antibody, in ADCP Effector Cell [FcγRIIa (H variant) /NFAT Reporter-Jurkat cells] co-cultured with BT-474.
The result is shown as fold induction of NFAT luciferase reporter. EC50 = 830 ng/ml

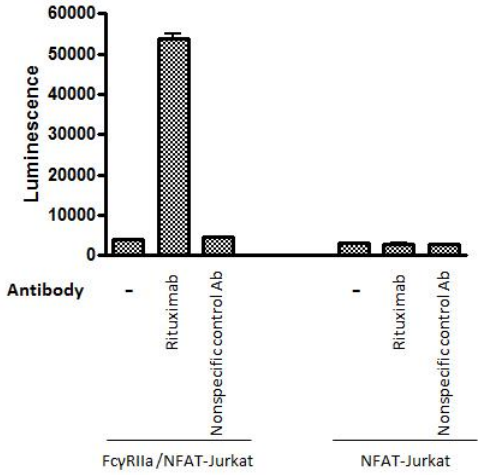


6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

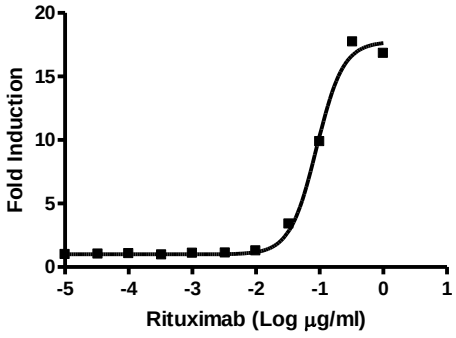
Figure 2. ADCP response to anti-CD20 (Rituximab), a chimeric IgG1 antibody drug, in ADCP Bioassay Effector Cell (FcγRIIa /NFAT Reporter-Jurkat cells), co-cultured with Raji cells.

Anti-CD20 (BPS, #71209), nonspecific control antibody, or assay medium (no antibody) was incubated with ADCP Bioassay Effector Cell (FcγRIIa /NFAT Reporter-Jurkat cells) or NFAT Reporter-Jurkat Cell (BPS, #60621), co-cultured with target cells (human B lymphocyte Raji cells). 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 ADCP response to Anti-CD20 (Rituximab)-induced NFAT luciferase reporter activity in ADCP Bioassay Effector Cells [FcγRIIa (H variant) /NFAT Reporter-Jurkat cells] co-cultured with Raji cells.



B. Dose response of Anti-CD20 in ADCP Bioassay Effector Cell (FcγRIIa /NFAT Reporter-Jurkat cells) co-cultured with Raji cells. The result is shown as fold induction of NFAT luciferase reporter. EC50 = 0.09 µg/ml

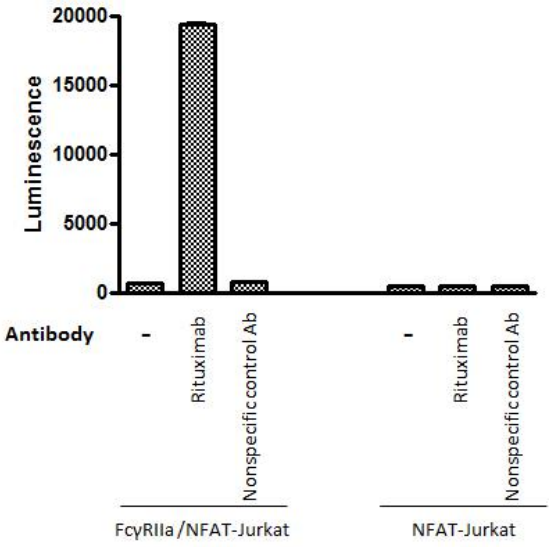


EC50= 0.09 µg/ml

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

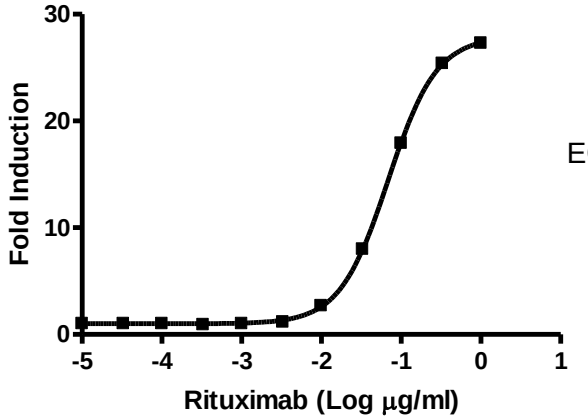
Figure 3. ADCP response to anti-CD20 (Rituximab) in ADCP Bioassay Effector Cells (FcγRIIa /NFAT Reporter-Jurkat cells), co-cultured with WIL2-S cells.
Anti-CD20 (BPS, #71209), nonspecific control antibody, or assay medium (no antibody) was incubated with ADCP Bioassay Effector Cell (FcγRIIa /NFAT Reporter-Jurkat cells) or NFAT Reporter-Jurkat Cells, co-cultured with 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 ADCP response to Anti-CD20 (Rituximab). Anti-CD20-induced NFAT luciferase reporter activity in ADCP Bioassay Effector Cells [FcγRIIa (H variant) /NFAT Reporter-Jurkat cells] co-cultured with WIL2-S cells.



6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

B. Dose response of Anti-CD20 in ADCP Bioassay Effector Cells (FcγRIIa /NFAT Reporter-Jurkat cells) co-cultured with WIL2-S cells.
The result is shown as fold induction of NFAT luciferase reporter.
EC50 = 0.069 µg/ml



EC50= 0.069 µg/ml

Related Products

Product	**Cat. #**	**Size**
ADCC Bioassay Effector Cell, F variant (Low Affinity)	60540	2 vials
ADCC Bioassay Effector Cell, R variant (high Affinity)	60541	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