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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

Description

Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line was generated from T Cell Receptor (TCR) Knockout NFAT Luciferase Reporter Jurkat Cell Line (#78556) by overexpression of human Vγ9Vδ2 TCR using lentiviral transduction (with Vγ9Vδ2 TCR Lentivirus, #78985).

This cell line was tested by flow cytometry and functionally validated with both anti-CD3 and TCR Vδ2 agonist antibodies.

Background

γδ TCRs (T cell receptors), αβ TCRs, and antibodies, result from gene rearrangements and offer the immune system the possibility to recognize several different types of antigens. γδ TCRs recognize antigens in a similar way to antibodies, being able to recognize full protein antigens and being independent on antigen binding to the MHC (major histocompatibility complex). Examples of such antigens include pyrophosphomonoesters from Mycobacterium tuberculosis and E. Coli, or glycoprotein I from Herpes simplex. γδ TCRs are cell type specific, with Vγ9Vδ2 being present in T cells and corresponding to about 5% of the T cell population in blood. Vγ9Vδ2 T cells are involved mostly in immune responses to pathogens and long-term modulation of inflammation, and can recognize non-peptide phosphor-antigens, alkylamines and synthetic aminobisphosphonates. Vγ9Vδ2 T cells are being studied for the treatment of solid tumors and hematological disorders and are becoming a highly promising cancer therapy. Further studies on how best to utilize Vγ9Vδ2 T cells, and methods to enhance their presence, will open new therapeutic avenues for cancer and infections.

Application(s)

- Screen Vγ9Vδ2 TCR agonist antibodies.
- Positive control for Vγ9Vδ2 TCR evaluation and optimization of experimental conditions.

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.

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2B	BPS Bioscience #79530
Growth Medium 2F	BPS Bioscience #79669
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78556
APC anti-human TCR Vγ9 Antibody	BioLegend #331310
TCR Vδ2 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotech #130-111-127
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
TCR V delta 2 Monoclonal Antibody (15D)	Life Technologies #TCR1732
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture plate, white, clear bottom	
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 by 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

Growth Medium 2B (BPS Bioscience #79530):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin.

Growth Medium 2F *(BPS Bioscience #79669):*

RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 µg/ml of Puromycin and 1 mg/ml Geneticin.

Media Used in Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, and 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 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 2, 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 2F**.**

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml with Growth Medium 2F. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~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 storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Protocols

A. T Cell Activation of Vvγ9Vvδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line by Anti-CD3 Agonist Antibody.

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Non-Coated Control", "Unstimulated Control" and "Test" wells.
- 5 ml of Jurkat cells at 4 x 10^5 cells/ml is enough for half of a 96-well plate.
- We recommend using TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line (#78556) as control.

1. Dilute anti-CD3 agonist antibody to 1 µg/ ml in PBS (200 µl/ well).

2. Coat a cell culture-treated, clear bottom, white 96-well plate with 200 µl/well of diluted anti-CD3 antibody for 1 hour at Room Temperature (RT) or overnight at 4°C. Leave a few non-coated wells to serve as "Non-Coated Control".

3. Remove the coating solution, wash the wells 3 times with 200 µl/well of PBS.

4. Harvest TCR Knockout NFAT-Luciferase Reporter Jurkat cells and Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat cells from Growth Medium 2B and Growth Medium 2F, respectively, by centrifugation and resuspend the cells in fresh Thaw Medium 2 and count.

5. Resuspend each cell line into 5 ml of fresh Thaw Medium 2 at a density of 4 x 10^5 cells/ml (100 µl/ well).

6. Add 100 µl of diluted TCR Knockout NFAT-Luciferase Reporter Jurkat cells to the wells reserved for the control cell line.

7. Add 100 µl of diluted Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat cells to the wells reserved for the test cell line.

8. Add 100 µl of Thaw Medium 2 to the "Unstimulated Control" wells (for measuring the basal luciferase activity) and "Non-Coated Control" wells of each cell line.

9. Incubate the plate at 37°C with 5% CO_2 overnight.

10. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

11. Incubate at RT for ~15 to 30 minutes.

12. Measure luminescence using a luminometer.

B. T Cell Activation of Vvγ9Vvδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line by a TCR Vδ2 agonist antibody.

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Unstimulated Control" and "Test" wells.
- 2.5 ml of Jurkat cells at 4 x 10^5 cells/ml is enough for half of a 96-well plate.
- We recommend using TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line (#78556) as control.

1. Harvest TCR Knockout NFAT-Luciferase Reporter Jurkat cells and Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat cells from Growth Medium 2B and Growth Medium 2F, respectively, by centrifugation and resuspend the cells in fresh Thaw Medium 2 and count.

2. Resuspend each cell line into 2.5 ml of fresh Thaw Medium 2 at a density of 4 x 10^5 cells/ml (50 µl/well).

3. Add 50 µl of diluted TCR Knockout NFAT-Luciferase Reporter Jurkat cells to the wells reserved for the control cell line.

4. Add 50 µl of diluted Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat cells to the wells reserved for the test cell line.

5. Dilute the Vγ9Vδ2 TCR agonist antibody with Thaw Medium 2 at 2-fold higher than the desired final concentration (50 µl/well).

6. Add 50 µl of diluted TCR Vδ2 agonist antibody to the "Test" wells of each cell line.

7. Add 50 µl of Thaw Medium 2 to the "Unstimulated Control" wells (for measuring the basal luciferase activity) of each cell line.

8. Incubate the plate at 37°C with 5% CO_2 for 5-6 hours.

9. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

10. Incubate at RT for ~15 to 30 minutes.

11. Measure luminescence using a luminometer.

Validation Data

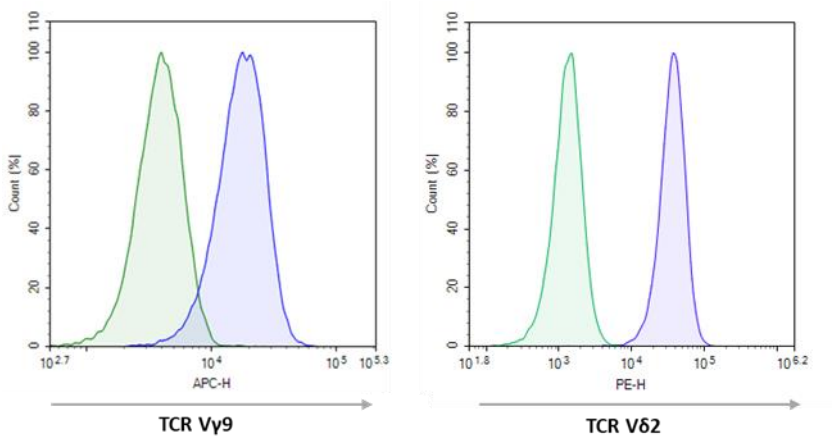

Figure 1: Expression of Vγ9Vδ2 TCR in Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line determined by flow cytometry.
Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat cells (blue) and TCR Knockout NFAT-Luciferase Reporter Jurkat (BPS Bioscience #78556) cells (green) were stained with both APC anti-human TCR Vγ9 Antibody (BioLegend #331310) (left) and TCR Vδ2 Antibody, anti-human, PE, REAfinity™ (Miltenyi Biotech #130-111-127) (right), and analyzed by flow cytometry. The y axis represents the % of cells. The x axis indicates fluorophore intensity.

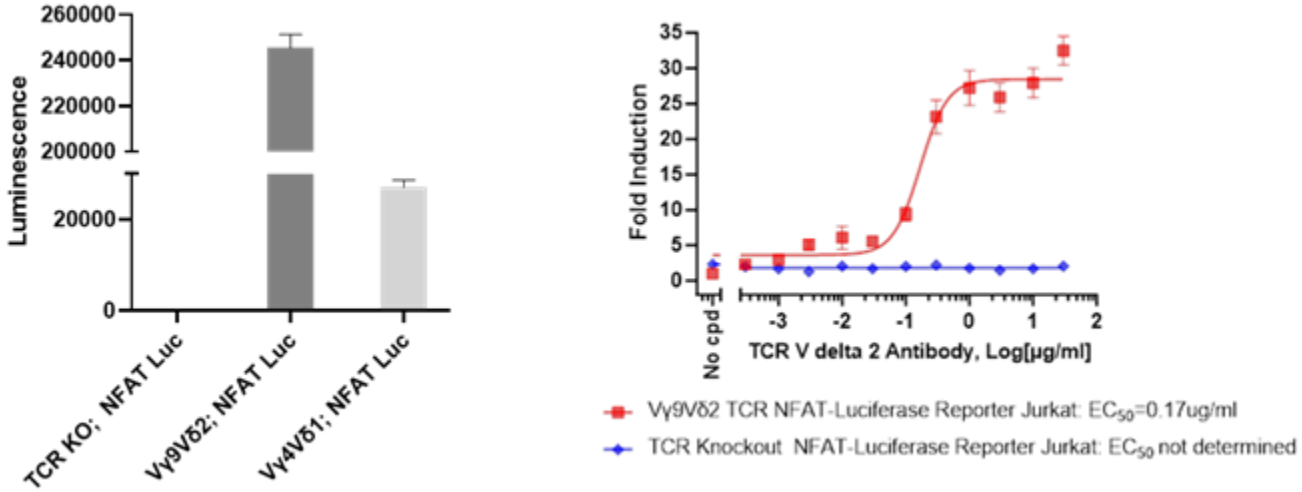

Figure 2: T Cell Activation of Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line by both anti-CD3 and TCR Vδ2 agonist antibody.
Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat and TCR KO NFAT Luciferase Reporter Jurkat (BPS Bioscience #78556) cells were stimulated overnight with 1 µg/ml of Anti-CD3 Agonist Antibody (BPS Bioscience #71274) (left) or 6 hours with TCR Vδ2 TCR agonist antibodies (Life Technologies #TCR1732) (right). Luciferase activity was measured with ONE-Step™ Luciferase Assay System, and the results are shown as fold induction of luminescence readings.

Data shown is representative.

Notes

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

License Disclosure

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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.

References

Allison T. and Garboczi D., 2002 *Molecular Immunology* 38 (14): 1051-1061.
Sawaisorn P., *et al.*, 2024 *Scientific Reports* 14: 1291.

Related Products

Products	*Catalog #*	*Size*
Vγ9Vδ2Vγ9Vδ2 TCR Lentivirus	78985	100 µl/2 x 500 µl
Vγ4Vδ1 TCR Lentivirus	78986	100 µl/2 x 500 µl
Vγ4Vδ1 TCR NFAT-Luciferase Reporter Jurkat Cell Line	82329	2 vials
TCR Knockout Jurkat Cell Line	78539	2 vials
$CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78757	2 vials
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	2 vials
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	2 vials

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