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Produktinformation

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

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Vγ4Vδ1 TCR NFAT-Luciferase Reporter Jurkat Cell Line was generated from the T Cell Receptor (TCR) Knockout NFAT Luciferase Reporter Jurkat Cell Line (#78556) by overexpression of human Vγ4Vδ1 TCR using lentiviral transduction (with Vγ4Vδ1 TCR Lentivirus #78986).

This cell line was functionally validated with a TCR Vδ1 agonist antibody.

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). γδ TCRs are cell type-specific, with Vγ4Vδ1 being present in γδTIL (tumor infiltrating lymphocytes) cells. Vδ1 expressing cells are found in mucosal and epithelial tissues and correspond to about 15% of the γδT cells present in PBMCs, with the % of γδ being only 5% of all the T cells. Vγ4Vδ1 cells can be activated by CD1 (cluster of differentiation 1) and respond to BTNL3 (butyrophilin-like 3), BTNL8, annexin A2 and A6. These cells can lead to tumor cell death by lysis. While most of the studies have been focusing on Vγ9Vδ2 T cells, a better understanding of the function and therapeutic potential of Vγ4Vδ1 T cells may open new avenues in cancer therapy.

Application(s)

- Screen Vγ4Vδ1 TCR agonist antibodies.
- Positive control for Vγ4Vδ1 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
TCR Vδ1 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotech #130-120-580
TCR V delta 1 Monoclonal Antibody (TS8.2)	Life Technologies #TCR1730
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture plate, white, clear bottom	
Luminometer	
PBS (Phosphate Buffer Saline)	

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 supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin.

Growth Medium 2F *(BPS Bioscience #79669):*
RPMI1640 medium 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 supplemented with 10% FBS, and 1% Penicillin/Streptomyci*n.*

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.

   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.

Assay Protocol

- 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. Appropriately dilute anti-TCR V delta 1 Monoclonal Antibody at the indicated concentration in PBS.

2. Coat a cell culture-treated, clear bottom, white 96-well plate with 200 µl/well of diluted anti-TCR V delta 1 Monoclonal 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 Vγ4Vδ1 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 Vγ4Vδ1 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 for 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.

Validation Data

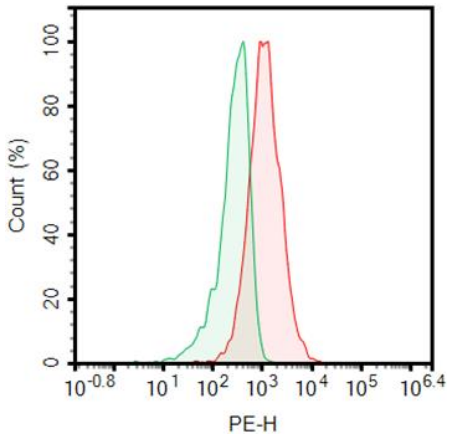

Figure 1: Expression of Vγ4Vδ1 TCR in Vγ4Vδ1 TCR NFAT-Luciferase Reporter Jurkat Cell Line determined by flow cytometry.
Vγ4vδ1 TCR NFAT-Luciferase Reporter Jurkat cells (red) and TCR Knockout NFAT-Luciferase Reporter Jurkat (BPS Bioscience #78556) cells (green) were stained with TCR Vδ1 Antibody, anti-human, PE, REAfinity™ (Miltenyi Biotech #130-120-580) and analyzed by flow cytometry. The y axis represents the % of cells. The x axis indicates fluorophore intensity.

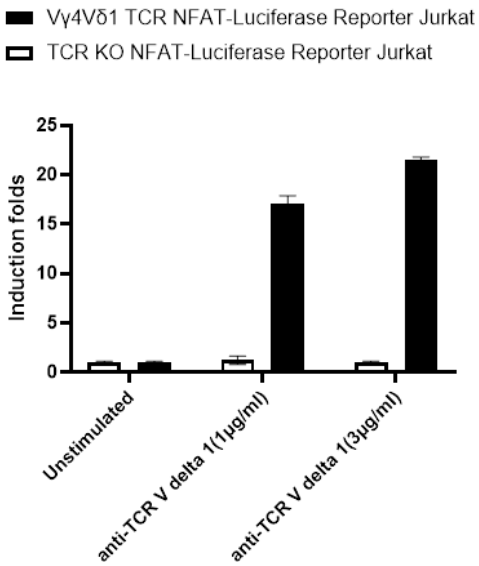

Figure 2: T Cell Activation of Vγ4Vδ1 TCR NFAT-Luciferase Reporter Jurkat Cell Line by a TCR Vδ1 agonist antibody.
Vγ4vδ1 TCR NFAT-Luciferase Reporter Jurkat and TCR Knockout NFAT Luciferase Reporter Jurkat (BPS Bioscience #78556) cells were stimulated overnight with anti-TCR V delta 1 Monoclonal Antibody (TS8.2) (Life Technologies #TCR1730). 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. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References

Allison T. and Garboczi D., 2002 *Molecular Immunology* 38 (14): 1051-1061.
Song Y., *et al.*, 2022 *Front Immunol* 13: 914839.

Related Products

Products	*Catalog #*	*Size*
Vγ9Vδ2 TCR Lentivirus	78985	100 µl/2 x 500 µl
Vγ4Vδ1 TCR Lentivirus	78986	100 µl/2 x 500 µl
Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line	82320	2 vials
TCR Knockout Jurkat Cell Line	78539	2 vials
$CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78757	2 vials
$CD4^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	82319	2 vials

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