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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- 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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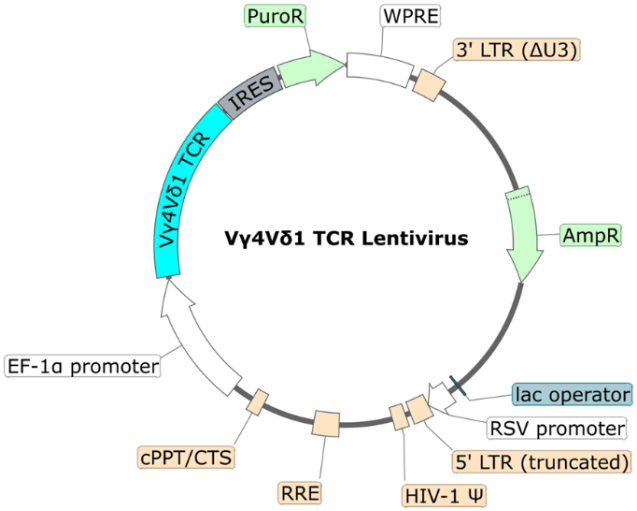
Description

Vγ4Vδ1 TCR Lentivirus are replication incompetent, HIV-based, VSV-G-pseudotyped lentiviral particles ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These viruses transduce a TCR (T Cell Receptor) which belongs to the Vγ4Vδ1 subtype of γδ TCRsd. The TCR γ chain and δ chain are linked by P2A. The lentiviruses also transduce a puromycin selection marker (Figure 1).

A



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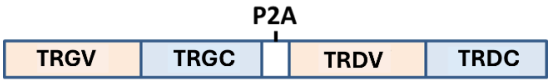

Figure 1. (A) *Schematic of the lenti-vector used to generate the Vγ4Vδ1 TCR Lentivirus and (B) diagram of the construct, showing the components of the Vγ4Vδ1 TCR.*

TRGV and TRGC correspond to the TCR *γ* chain variable and constant regions, respectively, whereas TRDV and TRDC correspond to the TCR *δ* chain variable and constant regions.

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

- Use to transduce cells with the goal of screening Vγ4Vδ1 TCR agonist antibodies.
- Use to transduce cells to be used as a positive control for Vγ4Vδ1 TCR evaluation and optimize experimental conditions.
- Generate Vγ4Vδ1 TCR expressing cell pools or stable cell lines, following puromycin selection.

Formulation
The lentiviruses were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer
≥2 x 10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage
Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the virus at -80°C for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety
The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied
These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems are all validated and optimized for use with this lentivirus and are highly recommended for best results.

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
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
TCR Vδ1 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotech #130-120-580
TCR V delta 2 Monoclonal Antibody (15D)	Life Technologies #TCR1732
TCR V delta 1 Monoclonal Antibody (TS8.2)	Life Technologies #TCR1730
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Lenti-Fuse Polybrene Viral Transduction Enhancer	BPS Bioscience #78939

Media Formulations
For best results, the use of BPS Bioscience validated and optimized media is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

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/Streptomycin.

Assay Protocol

- The following protocol was used to transduce a Jurkat cell line. The transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the reporter gene with puromycin prior to carrying out the reporter assays.
- The assay should include "Negative Control" (uncoated wells) and "Testing" wells.
- 1 ml of Jurkat cells at 2 x 10^5 cells/ml is enough for 12 wells of a 96-well plate.

Day 1:

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

2. Dilute cells to a density of 2 x 10^5/ml in Thaw Medium 2.

3. Mix 1 ml of the Jurkat cells with the appropriate amount of Vγ4Vδ1 TCR Lentivirus in a 1.5-ml Eppendorf tube.

   Note: The MOI may need to be optimized, we recommend a starting MOI of 5.

4. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to a final concentration of 8 µg/ml.

5. Gently mix and incubate the virus with the Jurkat cells for 20 minutes at Room Temperature (RT) in a tissue culture hood.

6. Centrifuge the virus/cell mixture for 30-120 minutes at 800 *x g* and 32°C (spinoculation).

7. Add the cells/virus mix from the spinoculation step to one well of a 6-well plate.

8. Add an additional 1.5 ml of Thaw Medium 2 to the well.

   Note: It is not necessary to remove the virus.

9. Incubate the cells at 37°C with 5% CO_2 for 48-66 hours.

Day 3:

1. The expression of TCR can be analyzed by flow cytometry. The transduced Jurkat cells are ready for assay development on day 3 or 4.

   Note: If the transduction efficiency is low, it may be necessary to initiate cell selection with puromycin on day 3.

2. Coat a cell culture-treated, clear bottom, white 96-well plate with 100 µl of control or agonist antibodies at 10 µg/ml in PBS (Phosphate Buffer Saline) at 4°C overnight. Leave a few non-coated wells as "Negative Control".

Day 4:

1. Wash all wells of the coated plate three times with PBS. The plate is ready to use.
2. Harvest the transduced Jurkat cells and resuspend the cells into 1.2 ml of fresh Thaw Medium 2.
3. Add 100 µl of cells to each well of the antibody-coated 96-well plate.
4. Incubate the plate at 37°C with 5% CO_2 for 5-6 hours.
5. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.
6. Incubate at RT for ~15 to 30 minutes.
7. Measure luminescence using a luminometer.

Validation Data

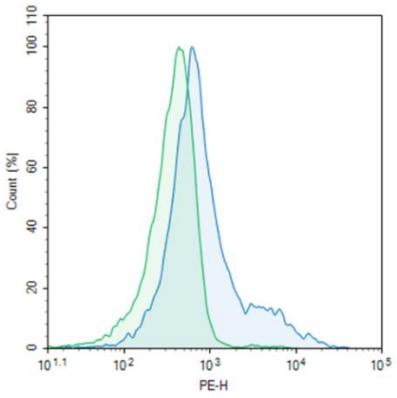

Figure 2. Analysis of the expression of Vγ4Vδ1 TCR in TCR Knockout NFAT-Luciferase Reporter Jurkat Cells transduced with the Vγ4Vδ1 TCR Lentivirus.
Approximately 100,000 TCR Knockout NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #78556) were transduced with Vγ4Vδ1 TCR Lentivirus by spinoculation at a MOI of 10. Transduced cells were selected with Growth Medium 2F and 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. Green, untransduced cells; Blue, Vγ4Vδ1 TCR transduced cells.

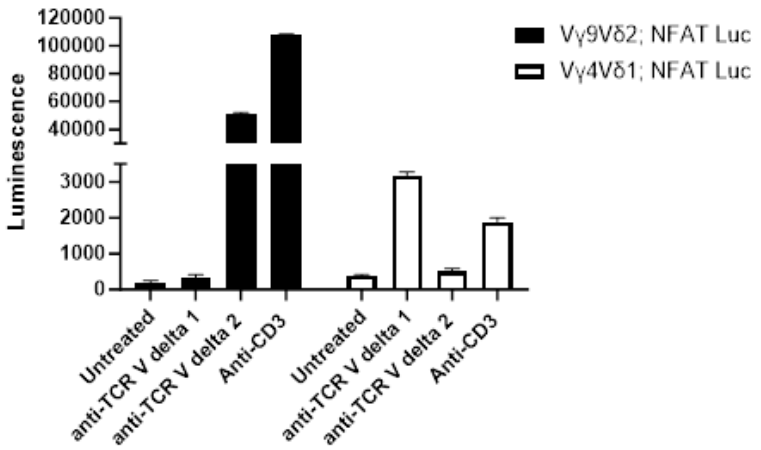

Figure 3. NFAT luciferase reporter activity in TCR Knockout NFAT-Luciferase Reporter Jurkat cells transduced with Vγ9Vδ2 TCR Lentivirus and Vγ4Vδ1 TCR Lentivirus and stimulated with agonist antibodies.

TCR Knockout NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #78556) were transduced with Vγ9Vδ2 TCR Lentivirus (BPS Bioscience #78985) or Vγ4Vδ1 TCR Lentivirus (BPS Bioscience #78986). Transduced cells were selected with Growth Medium 2F and stimulated with plate coated TCR V delta 1 Monoclonal Antibody (TS8.2) (Life Technologies #TCR1730), TCR V delta 2 Monoclonal Antibody (15D) (Life Technologies #TCR1732), and Anti-CD3 Agonist Antibody (BPS Bioscience #71274) for 5 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Cells transduced with Vγ4Vδ1 TCR Lentivirus can be activated by the Vδ1 antibody, but not the Vδ2 antibody, while cells transduced with Vγ9Vδ2 TCR Lentivirus can be activated by the Vδ2 antibody, but not the Vδ1 antibody. CD3 agonist antibody treatment was run in parallel as a positive control.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Notes

To generate a Vγ4Vδ1 TCR expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For 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

Version 042524