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Produktinformation

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

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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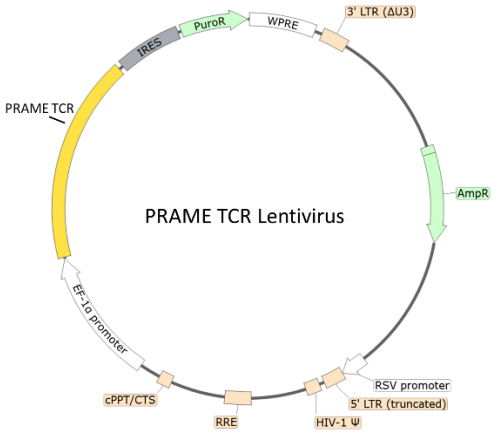
Description

PRAME-Specific 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 cells with a TCR (T cell receptor) that specifically recognizes the human antigen PRAME (Preferentially Expressed Antigen in Melanoma) peptide 425-433 (SLLQHLIGL), and in which the TCR α chain and β chain are linked by P2A. The lentiviruses also transduce a puromycin selection marker (Figure 1).

A



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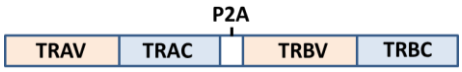

Figure 1. (A) *Schematic of the lenti-vector used to generate the PRAME-Specific TCR Lentivirus and (B) diagram of the construct, showing the components of the PRAME-specific TCR.*
TRAV and TRAC correspond to the TCR alpha chain variable and constant regions, respectively, whereas TRBV and TRBC correspond to the TCR beta chain variable and constant regions.

Background

PRAME (Preferentially Expressed Antigen in Melanoma) is a protein with a profile of expression in normal tissues highly restricted to testis, ovarium, and endometrium. However, it is found at high levels in several cancer types, such as melanoma, breast, and lung cancer. It is also found in cells of patients with AML (acute myeloid leukemia) and Hodgkin's lymphoma. Overexpression seems to block retinoic acid-mediated cell proliferation, differentiation, and apoptosis, contributing to tumorigenesis. Its expression pattern makes it an attractive target for immunotherapy. It is a membrane-bound protein, and it is thus a good target for TCR (T cell receptor)-T cells and anti-PRAME vaccines. Several clinical trials are ongoing and have demonstrated the clinical potential of targeting PRAME in melanoma, lung cancer and other solid tumors. Further studies into the functions of this protein will bring new clinical advances in cancer therapy.

Application

- Use as a positive control for PRAME TCR evaluation and optimize experimental conditions.
- Generate PRAME 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.

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 2C	BPS Bioscience #79592
Assay Medium 2D	BPS Bioscience #78755
$CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78757
T2 Cell Line	ATCC #CRL-1992
PRAME Peptide (425-433)	BPS Bioscience #78991
PE anti-human α/β T Cell Receptor Antibody	BioLegend #306707
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 Maintaining $CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line

Growth Medium 2C (BPS Bioscience #79592):

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin, 100 µg/ml Hygromycin B.

Media Required for Maintaining T2 Cell Line

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Media Required for Co-culture Assay

Assay Medium 2D (BPS Bioscience #78755):
RPMI 1640 medium supplemented with 1% FBS.

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 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 "Peptide Loaded" and "Unloaded Control" wells.

Day 1:

1. Harvest $CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells from Growth Medium 2C 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 PRAME-Specific 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 3-4:

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. For use in the following co-culture assay at day 4 prepare materials and conditions as follows:

   a) Preparation of Antigenic-Mimetic Peptides:
   Thaw the PRAME Peptide (425-433, amino acid sequence SLLQHLIGL) at RT.
   Dilute the peptide with Assay Medium 2D at a concentration that is 5-fold higher than the desired final concentration (20 µl/well).

   Note: The peptide stock was dissolved in DMSO to a concentration of 1 mM. The final DMSO concentration in the co-culture assay should not be >0.3%.

   b) Preparation of Antigen Presenting Cells (APCs):
   Harvest T2 cells (APC) from Thaw Medium 2 and resuspend the cells into Assay Medium 2D at a density of 5 x 10^5/ml.
   Add 40 µl of T2 cells into each well of a 96-well plate.
   Add 20 µl of diluted peptide to the "Peptide Loaded" wells.
   Add 20 µl of Assay Medium 2D to the "Unloaded Control" wells (for measuring the basal luciferase activity).

   c) Resuspend Jurkat cells into Assay Medium 2D at a density of 4 x 10^5/ml.
   Add 40 µl of TCR-transduced $CD8^+$ TCR knockout NFAT-Luciferase Reporter Jurkat cells into each well of the 96-well plate containing the APCs.

3. Incubate the plate containing the co-culture at 37°C with 5% CO_2 for 5-6 hours or overnight.

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

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

6. Measure luminescence using a luminometer.

Notes

To generate a PRAME 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.

Validation Data

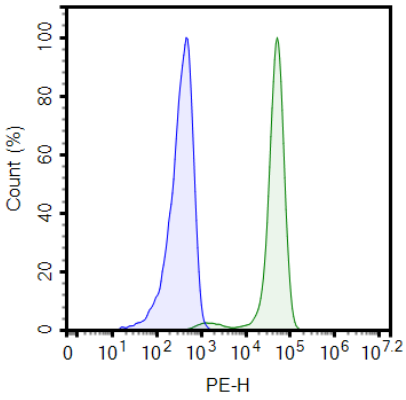

Figure 2. Expression of PRAME-specific TCR in Jurkat cells transduced with the PRAME-Specific TCR Lentivirus.
Approximately 100,000 CD8⁺ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with PRAME-Specific TCR Lentivirus by spinoculation at a MOI of 10. Sixty-six hours post-transduction, transduced cells (green) and non-transduced cells (blue) were stained with PE anti-human α/β T Cell Receptor Antibody (BioLegend #306707), and the expression of PRAME-specific TCR was analyzed by flow cytometry. The y axis represents the % of cells. The x axis indicates fluorophore intensity.

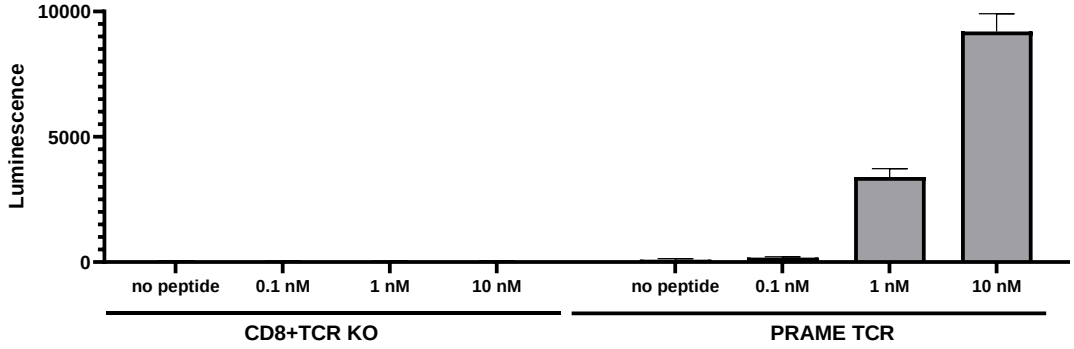

Figure 3. Jurkat T cell activation after transduction with PRAME-Specific TCR Lentivirus, using T2 cells as Antigen-presenting cells (APCs).
CD8⁺ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with PRAME-Specific TCR Lentivirus by spinoculation at a MOI of 10. Sixty-six hours post-transduction, cells were co-cultured with T2 cells (ATCC #CRL-1992) loaded with PRAME peptide (424-433, BPS Bioscience #78991) for 6 hours. Luciferase activity was measured, and the results are shown as raw luminescence readings. Untransduced CD8⁺ TCR Knockout NFAT-Luciferase Reporter cells, where no TCR is expressed, were run in parallel as a negative control.

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com.

References
Xu Y., *et al.*, 2020 *Cell Prolif.* 53(3):e12770.

Related Products

Products	*Catalog #*	*Size*
PRAME Peptide (394-402)	82307	100 µl
PRAME Peptide (432-440)	82306	100 µl
PRAME TCR $CD8^+$ NAFT-Luciferase Reporter Jurkat Cell Line	78997	2 Vials

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