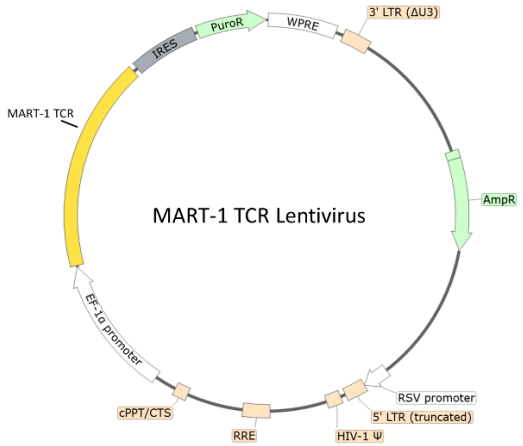
Description

The human MART-1-specific T Cell Receptor lentiviruses (clone DMF4) are replication incompetent, HIV-based, VSV-G-pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. These viruses transduce a human TCR (clone DMF4) that specifically recognizes antigen MART-1 (Melanoma-associated antigen recognized by T cells-1, or Melan-A), and in which the TCR α chain and β chain are linked by P2A (Figure 1). The lentiviruses also transduce a puromycin selection gene.

A



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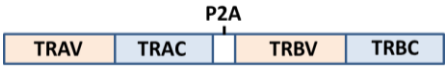

Figure 1. (A) Schematic of the lenti-vector used to generate the MART-1-specific TCR lentivirus and (B) Construct diagram showing expressed components of the MART-1-specific TCR. TRAV and TRAC correspond to the TCR alpha chain variable and constant regions, respectively, whereas TRBV and TRBC correspond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

Background

MART-1 is a differentiation antigen expressed at the surface of melanocytes. A peptide fragment of the protein is found bound to MHC complexes that are recognized by cytotoxic T cells. MART-1 is found in most skin cancers, including melanomas, and is used as a biomarker for diagnostic purposes. Since the expression of the protein is restricted to melanocyte containing tissues (skin and retina), and is not found in other tissues, MART-1 is an attractive target for cancer vaccines and adoptive cell therapy. MART-1 peptide 26-35 is a fragment commonly associated with MHC and recognized by T cell receptors.

Application

- Use as a positive control for MART-1 TCR evaluation and optimize experimental conditions.
- Generate MART-1 TCR stable cell line (puromycin resistant).

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

≥2x10^7 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. 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
TCR KO NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78556
$CD8^+$ TCR KO NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78757
T2 Cell Line	ATCC #CRL-1992
A375 Cells	ATCC #CRL-1619
NY-ESO-1 (157-165) Peptide	BPS Bioscience #78758
MART-1 (26-35) Peptide	BPS Bioscience #78759
MART-1 (Leu26-35, Leu27) Peptide	BPS Bioscience #78760
MART-1 (27-35) Peptide	BPS Bioscience #78761
APC MHC I Dextramer (HLA-A*02:01 SLLMWITQV)	Immudex #WB03247
PE anti-human α/β T Cell Receptor Antibody	BioLegend #306707
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Polybrene	Millipore Sigma #TR-1003-G

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 KO NFAT Luciferase Reporter 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 Cells

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 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.

1. Day 1: Harvest the $CD8^+$ TCR knockout NFAT Luciferase Reporter Jurkat cells from Growth Medium 2C by centrifugation and resuspend the cells in fresh Thaw Medium 2. Dilute the cells to a density of 2 x 10^5/ml in Thaw Medium 2. Mix 1 ml of the Jurkat cells and MART-1 TCR Lentivirus in a 1.5-ml Eppendorf tube at an MOI>10.

   Add polybrene to a final concentration of 8 µg/ml. Gently mix and incubate the virus with the Jurkat cells for 20 minutes at room temperature in a tissue culture hood.

2. Centrifuge the virus/cell mixture for two hours at 800 *x g* at 32°C (spinoculation). Add the cells/virus mix from the spinoculation step to one well of a 6-well plate. Add an additional 1.5 ml of Thaw Medium 2 to the well. It is not necessary to remove the virus. Incubate the cells at 37°C with 5% CO_2 for 48-66 hours.

   The expression of TCR can be analyzed by flow cytometry. The transduced Jurkat cells are ready for assay development on day 3 or 4. If the transduction efficiency is low, it may be necessary to perform cell selection with puromycin on day 3.

3. For use in the following co-culture assay at Day 4 prepare materials as follows:

   a) Preparation of Antigenic-mimetic Peptides:
   Thaw the MART-1 peptide of interest at room temperature. Dilute the peptide with Assay Medium 2D so that it is 5-fold higher than the desired final concentration.

   Note: The peptide stock was dissolved in DMSO at 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.

   Add 20 µl of diluted peptide to the "peptide stimulated" wells. Add 20 µl of Assay Medium 2D to the "unstimulated 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.

4. Incubate the co-culture plate at 37°C with 5% CO2 for 5-6 hours or overnight.

5. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Notes

To generate stable TCR-expressing cells, remove the growth medium 48-72 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells. For more information on how to determine the concentration of antibiotic required, see our FAQs resources "what is a kill curve?"

Validation Data

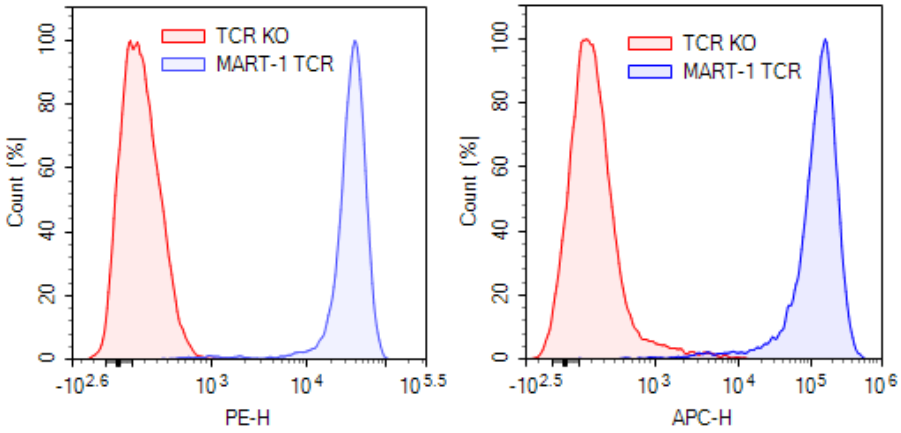

Figure 2. Expression of MART-1-specific TCR in Jurkat cells transduced with lentiviruses.
Approximately 100,000 $CD8^+$ TCR knockout NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with MART-1-specific TCR lentivirus (clone DMF4) (BPS Bioscience #78678) via spinoculation at a MOI of 10. After 48 hours of transduction, the cells were transferred into a medium containing 0.5 µg/ml of puromycin. After one week of antibiotic selection, the expression of MART-1-specific TCR (DMF4) was analyzed by flow cytometry. Left: cells were stained with PE conjugated anti-human TCR antibody (Biolegend #306707); right: cells were stained with APC-conjugated MHC-I Dextramer (HLA-A*02:01 ELAGIGILTV; Immudex#WB02162).

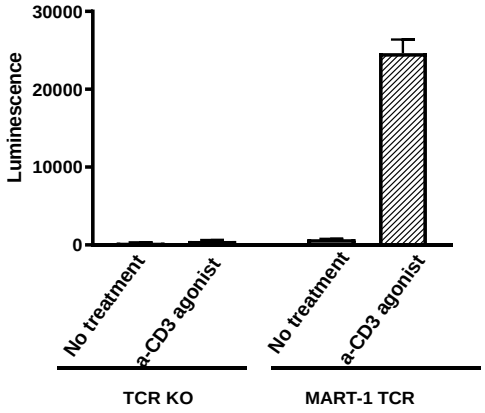

Figure 3. Expression of MART-1-specific TCR (clone DMF4) in TCR knockout NFAT Luciferase Reporter Cell Line confers responsiveness to anti-CD3 agonist and induces NFAT-dependent luciferase activity.
Approximately 20,000 TCR knockout NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78556)/well (96-well plate) were transduced with MART-1-specific TCR lentivirus (clone DMF4) (BPS Bioscience #78678) via spinoculation at a MOI of 10. After 66 hours of transduction, the medium was changed to Thaw Medium 2 (BPS Bioscience #60184). Cells were stimulated by transferring them to a 96-well plate pre-coated with anti-CD3 agonist antibody (BPS Bioscience #71274) at 1 µg/ml for 6 hours. The non-coated wells and the non-transduced cells were performed in parallel as controls. Results are shown as raw luminescence readings.

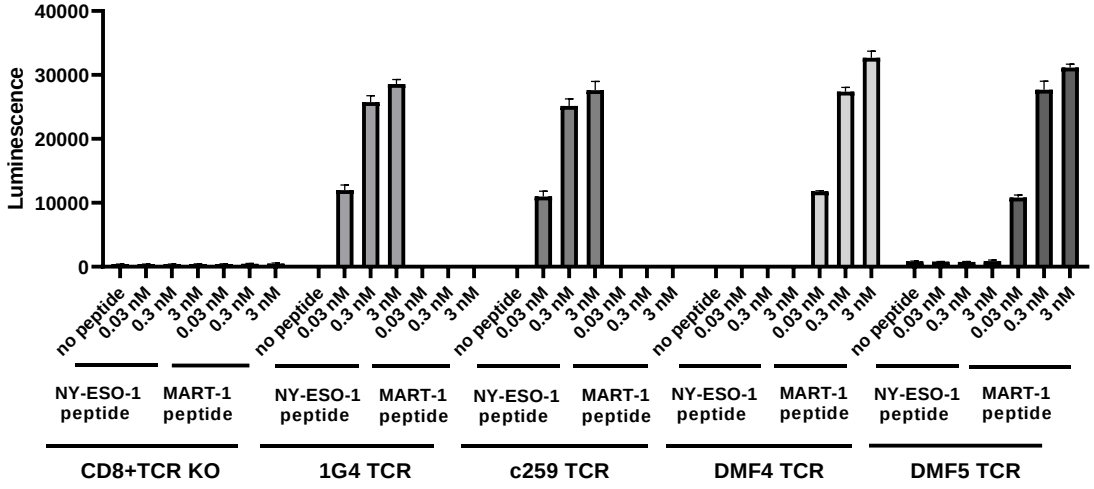

Figure 4. T Cell Activation using T2 cells as APC.
$CD8^+$ TCR knockout NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with lentiviruses expressing various TCRs via spinoculation at a MOI of 10. After 66 hours of transduction, the cells were co-cultured with T2 cells (ATCC #CRL-1992) loaded with NY-ESO-1 peptide (BPS Bioscience #78758) or with MART-1 peptide (BPS Bioscience #78760) for 6 hours. The luciferase assay was performed, and the results are shown as raw luminescence readings. Cells transduced with NY-ESO-1-specific TCR (clones 1G4, BPS Bioscience #78765,-and c259, BPS Bioscience #78766) can be activated by NY-ESO-1 peptide, but not MART-1 peptide, while cells transduced with MART-1-specific TCR (clones DMF4, BPS Bioscience #78678, and DMF5, BPS Bioscience #78679) can be activated by MART-1 peptide, but not NY-ESO-1 peptide. Un-transduced $CD8^+$ TCR Knockout NFAT Luciferase reporter cell line, where no TCR is expressed, was run in parallel as a negative control.

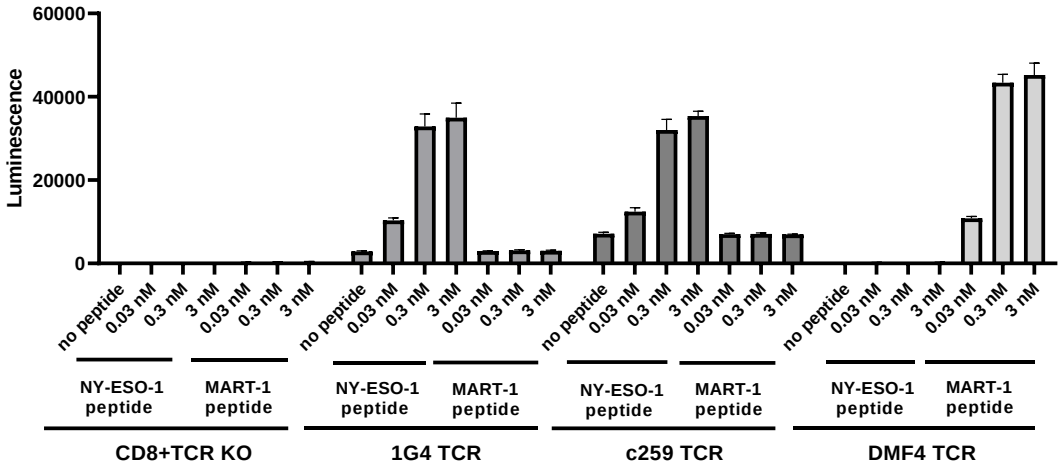

Figure 5. T Cell Activation using A375 cells as APC.
CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with lentiviruses expressing various TCRs via spinoculation at a MOI of 10. After 66 hours of transduction, the cells were co-cultured with A375 cells (ATCC # CRL-1619) loaded with NY-ESO-1 peptide (BPS Bioscience #78758) or MART-1 peptide (BPS Bioscience #78760) loaded for 6 hours. The luciferase assay was performed, and the results are shown as raw luminescence readings. Cells transduced with NY-ESO-1-specific TCR (clones 1G4, BPS Bioscience #78765,-and c259, BPS Bioscience #78766) can be activated by NY-ESO-1 peptide, but not MART-1 peptide, while cells transduced with MART-1-specific TCR (clone DMF4) (BPS Bioscience #78678) can be activated by MART-1 peptide, but not NY-ESO-1 peptide. Un-transduced CD8⁺ TCR Knockout NFAT Luciferase reporter cell line, where no TCR is expressed, was run in parallel as a negative control.

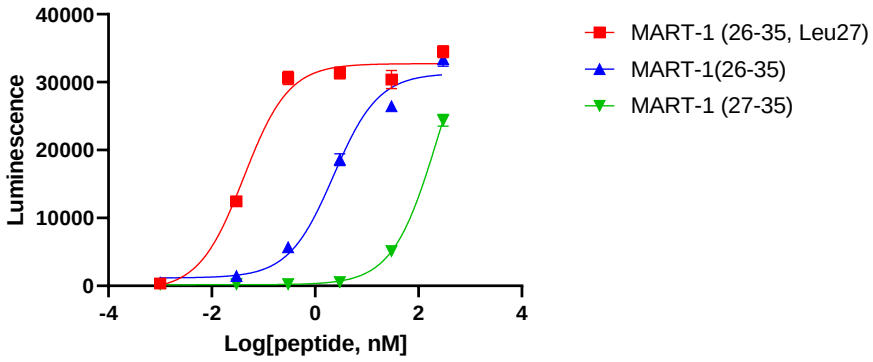

Figure 6. Antigenic Peptide Potency Ranking CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with lentiviruses expressing MART-1 TCR (Clone DMF4) (BPS Bioscience #78678) via spinoculation at a MOI of 10.
After 66 hours of transduction, the cells were co-cultured with different MART-1 peptide loaded T2 cells for 6 hours. The luciferase assay was performed, and the results are shown as raw luminescence readings. The native MART-1 decapeptide (26-35, EAAGIGILTV, BPS Bioscience #78759) is more efficiently recognized by MART-1 TCR (clone DMF4) transduced CD8⁺ TCR Knockout NFAT Luciferase Jurkat cells than the MART-1 peptide (27-35, AAGIGILTV, BPS Bioscience #78761), but has lower binding affinity and stability than the MART-1 (26-35, Leu27; ELAGIGILTV, BPS Bioscience #78760) analog.

Troubleshooting Guide

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

References

1. Chodon T. *et al.* Adoptative transfer of MART-2 T-cell receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma. *Clin Cancer Res.* (2014) 20 (9): 2457-65.
2. Rohaan M. *et al.*, MART-1 TCR gene-modified pheripheral blood T Cells for the treatment of metastic melanoma: a phase I/IIa clinical trial. Immunooncol *Technol.* (2022) Jun18: 15.
3. Kropp KN. E*t al.*, A bicistronic vector backbone for rapid seamless cloning and chimerization of αβT-cell receptor sequences. *PLOS One* (2020) 15(9): e0238875.

Related Products

Products	*Catalog #*	*Size*
NY-ESO-1-Specific TCR Lentivirus (Clone 1G4)	78675	100 µl/2 x 500 µl
NY-ESO-1-Specific TCR Lentivirus (Clone c259)	78676	100 µl/2 x 500 µl
MART-1-Specific TCR Lentivirus (Clone DMF5)	78679	100 µl/2 x 500 µl
NY-ESO-1 Peptide (157-165)	78758	100 µl
MART-1 Peptide (26-35)	78759	100 µl
MART-1 Peptide (26-35, Leu 27)	78760	100 µl
MART-1 Peptide (27-35)	78761	100 µl