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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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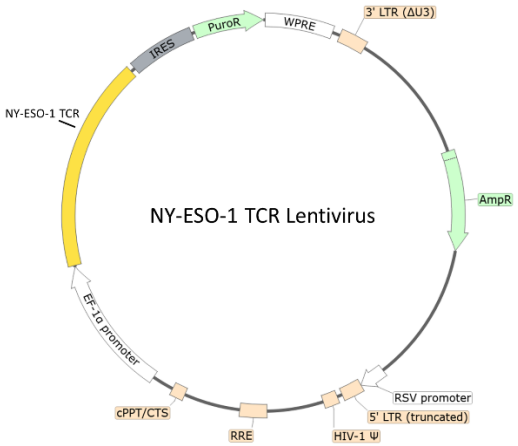
Description

The human NY-ESO-1-specific T Cell Receptor (TCR) lentiviruses (clone 1G4) 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 cells with a human TCR (clone 1G4) that specifically recognizes antigen NY-ESO-1 (New York esophageal squamous cell carcinoma 1), 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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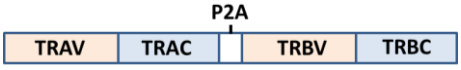

Figure 1. (A) Schematic of the lenti-vector used to generate the NY-ESO-1-specific TCR lentivirus and (B) Construct diagram showing expressed components of the NY-ESO-1-specific TCR. TRAV and TRAC correspond to the TCR alpha chain variable and constant regions, respectively, whereas TRBV and TRBC correspond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

Background

NY-ESO-1 (New York esophageal squamous cell carcinoma 1, also known as Cancer/testis antigen 1, or CTAG1B), is an important tumorigenic marker present in malignant cells. Normally expressed only in embryonic testis, this highly immunogenic protein is not usually found in normal tissues, but is re-expressed in multiple myeloma, non-small cell lung carcinoma, and breast and ovarian cancer, making it a promising candidate antigen for cancer immunotherapy. Several NY-ESO-1-directed therapies are being developed including cancer vaccines, anti-NY-ESO-1 adoptive cell therapy, and NY-ESO-1-specific TCR-T cell therapy in combination with checkpoint inhibitors.

Application

- Use as a positive control for NY-ESO-1 TCR evaluation and optimize experimental conditions.
- Generate NY-ESO-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

≥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. 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 (Leu26-35, Leu27) Peptide	BPS Bioscience #78760
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 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 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 $CD8^+$ TCR knockout/NFAT Luciferase Reporter Jurkat cells from Growth Medium 2C by centrifugation and resuspend the cells in fresh Thaw Medium 2. Dilute cells to a density of 2 x 10^5/ml in Thaw Medium 2. Mix 1 ml of the Jurkat cells and NY-ESO-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 NY-ESO-1 peptide (157-165, amino acid sequence SLLMWITQV) 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 plate.

   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 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

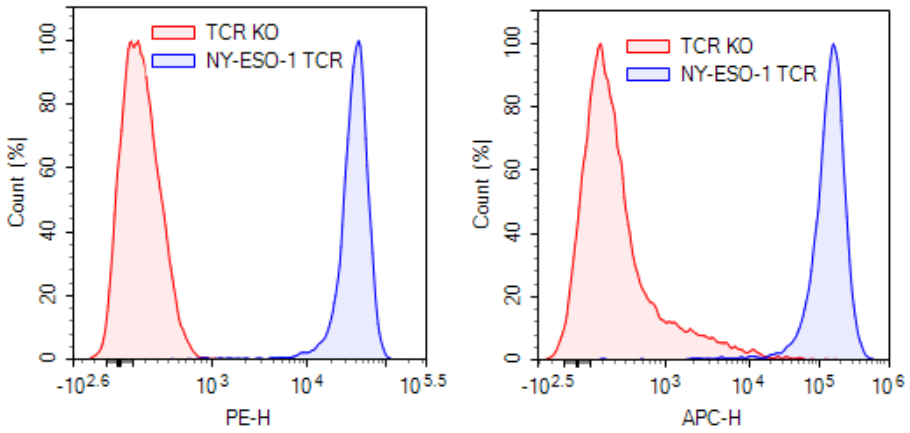

Figure 2. Expression of NY-ESO-1-specific TCR in Jurkat cells transduced with the lentivirus.
Approximately 100,000 $CD8^+$ TCR knockout/NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with NY-ESO-1-specific TCR lentivirus (clone 1G4; BPS Bioscience #78676) via spinoculation 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 NY-ESO-1-specific TCR in clone 1G4 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 SLLMWITQV; Immudex#WB03247)

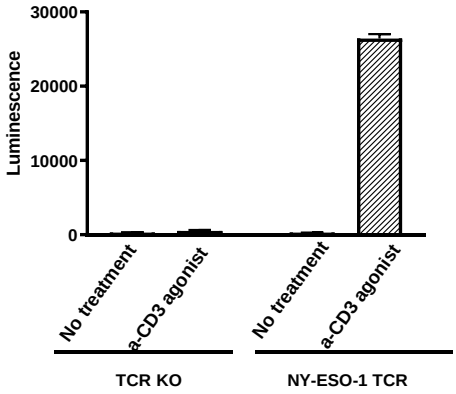

Figure 3. Expression of NY-ESO-1-specific TCR (clone 1G4) in TCR knockout/NFAT Luciferase Reporter Cell Line confers responsiveness to anti-CD3 agonist and induces NFAT-dependent luciferase activity.
Approximately 20,000 TCR knockout/NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78556)/well (96-well plate) were transduced with NY-ESO-1-specific TCR lentivirus (clone 1G4; BPS Bioscience #78675) via spinoculation at a MOI of 10. After 66 hours of transduction, the medium was changed to Thaw Medium 2. 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.

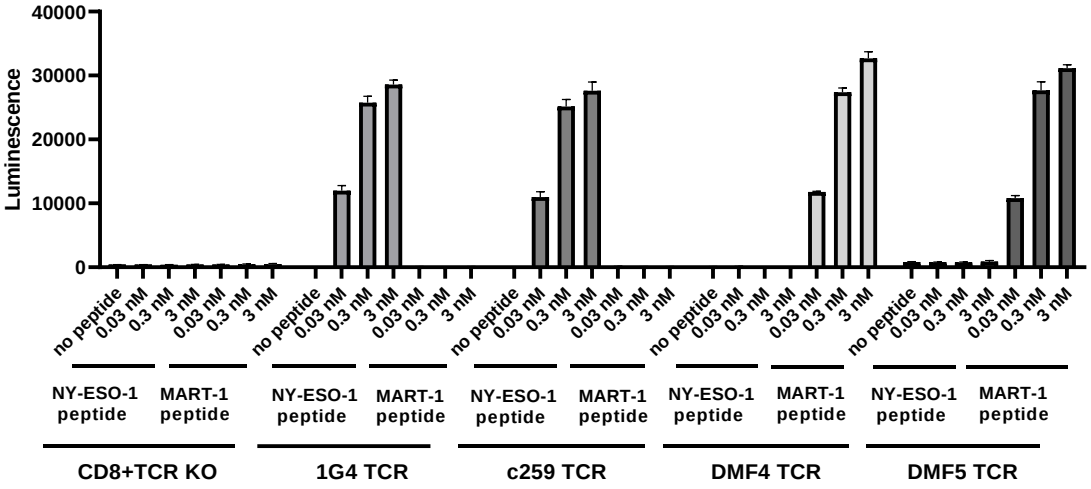

Figure 4. T Cell Activation using T2 cells as APC.
CD8$^+$ TCR knockout/NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with lentiviruses expressing various forms of TCR via spinoculation at a MOI of 10. After 66 hours of transduction, the cells were co-cultured with T2 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.

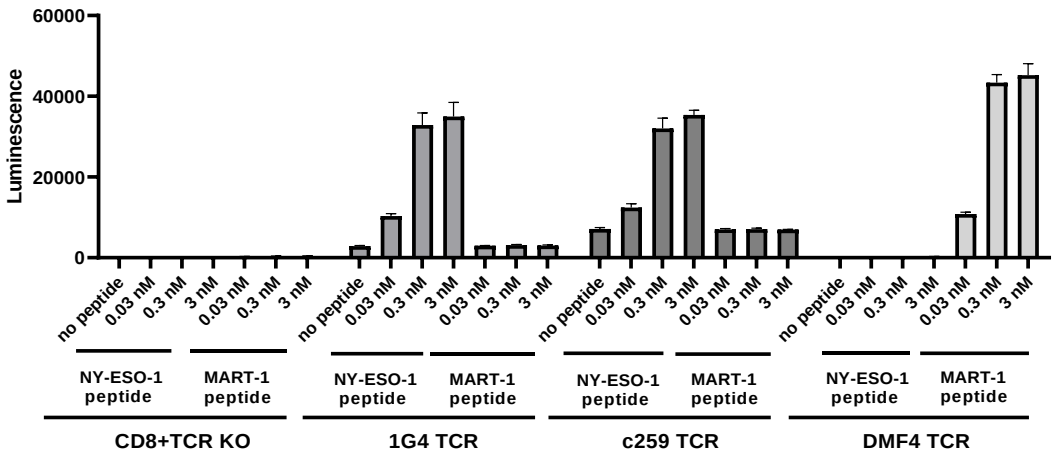

Figure 5. T Cell Activation using A375 cells as APC.
CD8⁺ TCR knockout/NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with lentiviruses expressing various forms of TCR via spinoculation at a MOI of 10. After 66 hours of transduction, the cells were co-cultured with A375 cells (ATCC #CRL-1619) loaded with NY-ESO-1 peptide (BPS Bioscience #78758) or MART-1 peptide (BPS Bioscience #78760) 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.

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

References
1. Thomas R. *et al.*, NY-ESO-1 Based Immunotherapy of Cancer: Current Perspectives. *Front. Immunol.* (2018) 9: 00947.
2. Raza A. *et al.*, Unleashing the immune response to NY-ESO-1 cancer testis antigen as a potential target for cancer immunotherapy. *J. of Translational Medicine* (2020) 140.
3. Kropp KN. *et 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 c259)	78676	100 µl/2 x 500 µl
MART-1-Specific TCR Lentivirus (Clone DMF4)	78678	100 µl/2 x 500 µl
MART-1-Specific TCR Lentivirus (Clone DMF5)	78679	100 µl/2 x 500 µl
MART-1 Peptide (27-35)	78761	100 µl
MART-1 Peptide (26-35)	78759	100 µl