Description

The NY-ESO-1 (c259) TCR-T Cells are generated by high-titer lentiviral transduction of human primary $CD4^+$ and $CD8^+$ T cells with NY-ESO-1-Specific TCR Lentivirus (Clone c259) (#78676). These ready-to-use TCR (T cell receptor) -T cells express the human TCR clone c259, that specifically recognizes the antigen NY-ESO-1 (New York esophageal squamous cell carcinoma 1).

These TCR-T cells have been validated by flow cytometry (to determine the TCR expression) and co-culture assays (IFN-γ staining and degranulation).

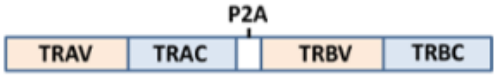

Figure 1: Construct diagram showing components of the NY-ESO-1-specific TCR expressed in NY-ESO-1 (c259) TCR-T Cells.

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 (NSCLC), 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 (s)

- Positive control in NY-ESO-1 TCR-T development.
- Screen modulators of NY-ESO-1 TCT-T driven cytotoxicity.
- Design and optimize co-culture bioassays for NY-ESO-1-specific TCR cell evaluation.

Materials Provided

Components	Format
One vial of frozen cells	Each vial contains 5 x 10^6 cells in 1 ml of CryoStor® CS10 (Stemcell Technologies # 100-1061)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

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.

Materials Required but Not Supplied

These materials are not supplied with the TCR-T cells but are necessary for cell culture and for the cellular assays described below. BPS Bioscience's reagents are validated and optimized for use with these cells and are highly recommended for best results.

Name	Ordering Information
Human Interleukin-2 Recombinant	BPS Bioscience #90184
Human CD3/CD28/CD2 T Cell Activator	Stemcell Technologies #10970
T2 Cell Line	ATCC #CRL-1992
MAGE-A4 Peptide (230-239)	BPS Bioscience #78966
NY-ESO-1 Peptide (157-165)	BPS Bioscience #78758
APC MHC I Dextramer (HLA-A*02:01 SLLMWITQV)	Immudex #WB03247
Untransduced T Cells (Negative Control for TCR-T)	BPS Bioscience #78989
Anti-CD8 Antibody, FITC	BPS Bioscience #102224
APC anti-human CD107a (LAMP-1) Antibody	BioLegend #328620
APC anti-human IFN-γ	BioLegend #986702
Monensin sodium	Medchem #HY-N0150
Brefeldin A	Medchem #HY-16592
Cell Staining Buffer	BioLegend #420201
Fixation Buffer	BioLegend #420801
Permeabilization Wash Buffer	BioLegend #421002

Recommended TCR-T Cell Medium: TCellM™ (#78753) supplemented with 10 ng/ml Interleukin-2 (#90184).

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed TCR-T Cell Medium.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. 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 TCR-T Cell Medium.

3. Transfer the resuspended cells to a T25 flask.

4. If desired culture the cells at 37°C with 5% CO_2 for 24-48 hours.

Cell Culture

1. Centrifuge the cells gently at 300 *x g* for 5 minutes.

2. Resuspend in fresh TCR-T Cell Medium.

3. Continue to culture the cells at 37°C with 5% CO_2.

4. Do not allow the cell density to exceed 2 x 10^6 cells/ml. Transfer the cells in larger culture vessels and add fresh medium when the density reaches 2 x 10^6 cells/ml.

It is recommended that NY-ESO-1 TCR-T cells are not activated for expansion after thawing. Since these are primary cells that have already been cultured, the extent of expansion is not predictable. Perform the cytotoxicity assay as soon as possible to avoid T cell exhaustion. NY-ESO-1 TCR-T Cells should not be in culture for more than 5 days. It is not recommended to freeze the cells again.

Validation

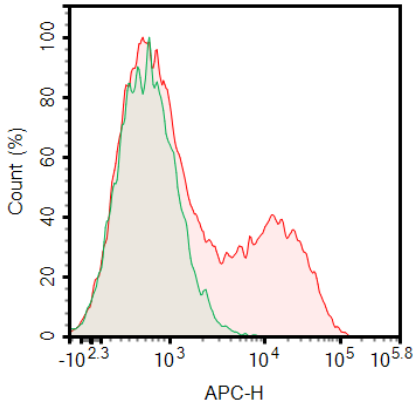

Figure 2: Expression of NY-ESO-1 (c259) TCR in NY-ESO-1 (c259) TCR-T Cells.
NY-ESO-1 (c259) TCR-T cells (red) and Untransduced T cells (green) were thawed and cultured for 24 hours. ~50,000 cells were stained with APC MHC I Dextramer (HLA-A*02:01 SLLMWITQV) (Immudex #WB03247) and analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates APC-intensity.

Functional Validation
The following experiments are examples of co-culture assays used to evaluate the cytotoxicity potential of NY-ESO-1 TCR-T cells by **A**)Intracellular cytokine (IFN- γ) staining and a **B)** Degranulation assessment.

A. TCR-T cell intracellular cytokine (IFN-γ) staining

- This assay described was performed in a 24-well plate with a 500 µl volume. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- We recommend using Untransduced T Cells as negative control.
- We recommend including the following controls: “No T2 Cells”, “No Peptide” and “MAGE-A4 Peptide” (as irrelevant peptide control).

1. NY-ESO-1 (c259) TCR-T cells and Untransduced T Cells were thawed and cultured for 24 hours in TCR-T Cell Medium according to the protocol in the “Cell Culture Protocol” Section.

2. Co-culture 5 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells with 1 x 10^5 T2 cells pulsed with 1 µM of NY-ESO-1 peptides, in TCR-T Cell Medium, for the “Test Condition”.

3. Co-culture 5 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells with 1 x 10^5 T2 cells pulsed with 1 µM of MAGE-A4 peptides, in TCR-T Cell Medium, for the "MAGE-A4 Peptide" (irrelevant peptide control).

4. Co-culture 5 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells with 1 x 10^5 T2 cells, in TCR-T Cell Medium, for the "No Peptide" condition.

5. 5 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells were kept without T2 cells, as "No T2 Cells" condition.

6. Incubate the cells at 37°C in 5% CO_2 for 6 hour.

   Optional: Add 2 µM of Monensin and 3 µM of Brefeldin A to each well 1 hour after the co-culturing was initiated, to aid with intracellular cytokine staining, and incubate the cells *at 37°C in 5% CO_2 for another 5 hours.*

7. Cells were washed twice with Cell Staining Buffer and stained with Anti-human CD8-FITC for 30 minutes at RT.

8. Cells were washed twice with Cell Staining Buffer and fixed for intracellular staining using the Fixation/Permeabilization Buffers according to the manufacturer's instructions.

9. Cells were analyzed by flow cytometry.

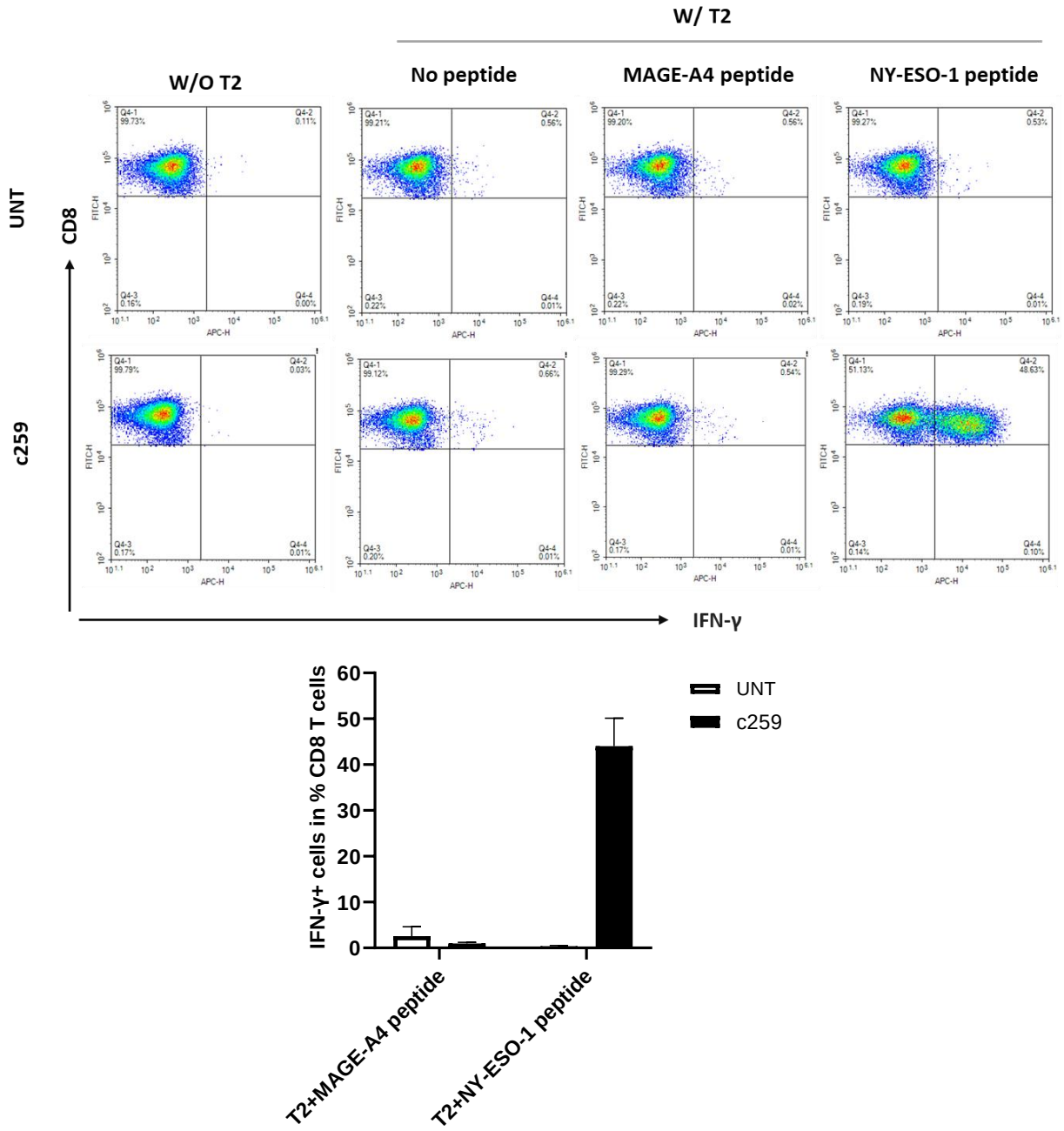

Figure 3: Flow cytometry assessment of IFN-γ cytokine production in NY-ESO-1 (c259) TCR-T Cells co-cultured with T2 cells, pulsed with different peptides.

NY-ESO-1 (c259) TCR-T cells (c259) and Untransduced T cells (UNT) were either co-cultured with NY-ESO-1 antigen-pulsed T2 cells or with antigen-negative (no peptide or irrelevant peptide) T2 cells for 6 hours. T cells were also cultured in absence of T2 cells as control. Cells were stained with Anti-CD8$^+$ Antibody, FITC (BPS Bioscience #102224) and stained intracellularly with APC anti-human IFN-γ (BioLegend #986702) and analyzed by flow cytometry. Top Panel: The x axis represents APC intensity, while the y axis shows FITC intensity. Bottom Panel: Quantification of % of CD8$^+$IFN-γ$^+$ cells.

B. Degranulation Assay

- This assay described was performed in a 96-well plate with a 100 µl volume. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- We recommend using Untransduced T Cells as negative control.
- We recommend including the following controls: "No T2 Cells", "No Peptide" and "MAGE-A4 Peptide" (as irrelevant peptide control).

1. NY-ESO-1 (c259) TCR-T cells and Untransduced T Cells were thawed and cultured for 24 hours in TCR-T Cell Medium according to the protocol in the "Cell Culture Protocol" Section.

2. Co-culture 1 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells with 2 x 10^4 T2 cells pulsed with 1 µM of NY-ESO-1 peptides, in TCR-T Cell Medium, and 5 µl of APC anti-human CD107a (LAMP-1) Antibody for the "Test Condition".

3. Co-culture 1 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells with 2 x 10^4 T2 cells pulsed with 1 µM of MAGE-A4 peptides, in TCR-T Cell Medium, and 5 µl of APC anti-human CD107a (LAMP-1) Antibody for the "MAGE-A4 Peptide" (irrelevant peptide control).

4. Co-culture 1 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells with 2 x 10^4 T2 cells, in TCR-T Cell Medium, and 5 µl of APC anti-human CD107a (LAMP-1) Antibody for the "No Peptide" condition.

5. 5 x 10^5 Untransduced T or NY-ESO-1 (c259) TCR-T cells were kept without T2 cells, as "No T2 Cells" condition. Add 5 µl of APC anti-human CD107a (LAMP-1) Antibody.

6. Incubate the cells at 37°C in 5% CO_2 for 6 hour.

   Optional: Add 2 µM of Monensin and 3 µM of Brefeldin A to each well 1 hour after the co-culturing was initiated, to aid with surface CD107a staining and incubate the cells at 37°C in 5% CO_2 for another 5 hours.

7. Cells were washed twice with Cell Staining Buffer and stained with Anti-Human CD8, FITC for 30 minutes at RT.

8. Cells were analyzed by flow cytometry.

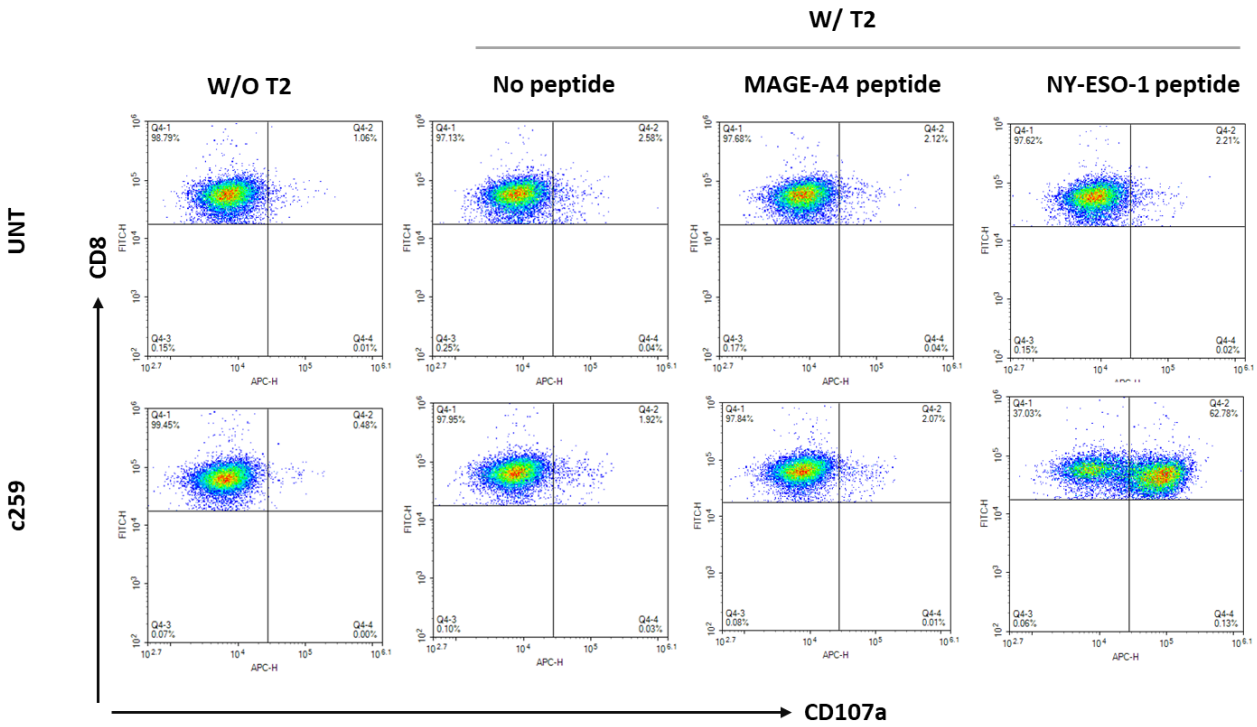

Figure 4: Flow cytometry assessment of CD107a degranulation in NY-ESO-1 (c259) TCR-T Cells co-cultured with T2 cells pulsed with different peptides.
NY-ESO-1 (c259) TCR-T cells (c259) and Untransduced T cells (UNT) were either co-cultured with NY-ESO-1 antigen-pulsed T2 cells or with antigen-negative (no peptide or irrelevant peptide) T2 cells and APC anti-human CD107a (LAMP-1) Antibody (BioLegend #328620) for 6 hours. T cells were also cultured in absence of T2 cells as control. Cells were stained with Anti-CD8$^+$ Antibody, FITC (BPS Bioscience #102224) and analyzed by flow cytometry. The x axis represents APC intensity, while the y axis shows FITC intensity.

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

References

Thomas R., *et al.*, 2018 *Front. Immunol.* 9: 00947.
Raza A., *et al.*, 2020 *J. of Translational Medicine* 140.
Kropp KN., *et al.*, 2020 *PLOS One* 15(9): e0238875.

Warnings
Donors have been screened and determined negative for:

- Hepatitis B (anti-HBc EIA, HBsAg EIA)
- Hepatitis C (anti-HCV EIA)
- Human Immunodeficiency Virus (HIV-1/HIV-2 plus O)
- Human T-Lymphotropic Virus (HTLV-I/II)
- HIV-1/HCV/HBV
- West Nile Virus
- Trypanasoma cruzi

Note: Testing cannot guarantee that any sample is completely virus-free. These cells should be treated as potentially infectious and appropriate biological safety level 2 (BSL-2) precautions should be used.

Troubleshooting Guide
Visit Cell Line FAQs for more information.
For further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
NY-ESO-1-Specific TCR Lentivirus (Clone c259)	78676	100 µl/500 µl x 2
NY-ESO-1-Specific TCR Lentivirus (Clone 1G4)	78675	100 µl/500 µl x 2
NY-ESO-1-Specific TCR (Clone 1G4) CD8 NFAT-Luciferase Reporter Jurkat Cell Line	78769	2 vials
NY-ESO-1-Specific TCR (Clone c259) CD8 NFAT-Luciferase Reporter Jurkat Cell Line	78771	2 vials
MAGE-A1-Specific TCR Lentivirus (Clone 1367)	78934	100 µl/500 µl x 2

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