# Description

The Untransduced T Cells (NY-ESO-1 TCR-T Negative Control) were produced by mock lentiviral transduction of human primary CD4<sup>+</sup> and CD8<sup>+</sup> T cells. These cells are subjected to comparable manipulations as TCR (T cell receptor)-T cells: activation, spinoculation (without lentivirus), and antigen specific stimulation. These T cells are designed as negative controls in experiments using lentivirus-transduced TCR-T cells, such as NY-ESO-1 (c259) TCR-T Cells (BPS Bioscience #78990).

### **Application**

Negative control for lentivirus-transduced NY-ESO-1 TCR-T cells.

### **Materials Provided**

Components	Format
One vial of frozen cells	Each vial contains 5 x 10° 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.

**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<sub>2</sub> for 24-48 hours.

### Cell Culture

- 1. Centrifuge the cells gently at 300 x g for 5 min.
- 2. Resuspend in fresh TCR-T Cell Medium.
- 3. Continue to culture the cells at 37°C with 5% CO<sub>2</sub>.



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

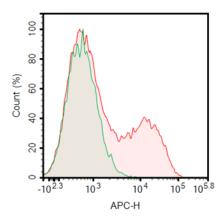


Figure 1: Expression of NY-ESO-1 (c259) TCR in NY-ESO-1 (c259) TCR-T Cells and Untransduced T Cells (NY-ESO-1 TCR-T Negative Control).

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.

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

#### 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 (c259) TCR-T Cells	78990	1 vial
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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