

Description

The Untransduced T Cells (MAGE-A4 TCR-T Negative Control) were produced by mock lentiviral transduction of human primary CD4⁺ and CD8⁺ 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 a negative control in experiments using lentivirus-transduced TCR-T cells, such as MAGE-A4 TCR-T Cells (#82390).

Background

MAGE (melanoma associated antigen) proteins are CT (cancer testis) antigens, and there are about 60 proteins in the MAGE family that can be subdivided into type I (present only on the X-chromosome, MAGE-A, B and C) and type II (MAGE D-L and necdin). Under normal conditions they are mostly found in the testis and placenta. They are found at high levels in several cancer types, such as melanoma, brain, and breast cancer, and are involved in the development of resistance to chemotherapy, cell motility and cell survival. Expression of MAGE proteins tends to correlate with a poor prognosis. They are intracellular proteins, with MAGE-A4 being found in the cytosol and nucleus, making them poor targets for strategies such as CAR-T cell therapy. MAGE proteins are degraded in the proteasome, and the peptides created can then be found on the cell membrane in combination with MHC (major histocompatibility complex) I. The presentation on the cell surface in this form makes them an attractive target for TCR (T cell receptor)-T cell therapy. In 2024, the first MAGE-A4 TCR engineered cell therapy for advanced synovial sarcoma was approved by the Food and Drug Administration (FDA).

Application

Negative control for lentivirus-transduced TCR-T cells expressing MAGE-A4.

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.

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 content 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% CO₂ 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% CO₂.
4. Do not allow the cell density to exceed 2 x 10⁶ cells/ml. Transfer the cells in larger culture vessels and add fresh medium when the density reaches 2 x 10⁶ cells/ml, at a minimum of 0.5 x 10⁶ cells/ml.



It is not recommended that the cells are 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. Untransduced T Cells should not be in culture for more than 5 days. It is not recommended to freeze the cells again.

Validation

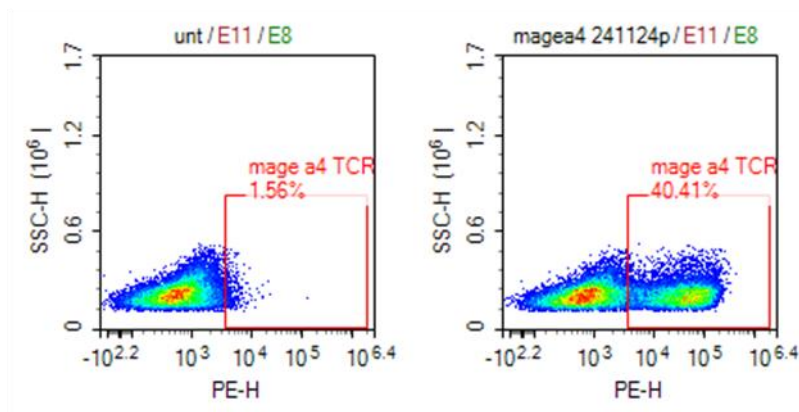


Figure 1: Expression of MAGE-A4 TCR in MAGE-A4 TCR-T Cells and in Untransduced T cells assessed by flow cytometry.

Untransduced T cells (left) and MAGE-A4 TCR-T cells (#82390) (right) were thawed and cultured for 24 hours. ~50,000 cells were stained with PE-labeled MHC I Dextramer (HLA-A*02:01 GYDGREHTV) (Immudex #WB03578) and analyzed by flow cytometry. The y axis represents the side scatter height, while the x axis indicates PE-intensity.

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

References

Kropp KN., *et al.*, 2020 *PLOS One* 15(9): e0238875.
 Caballero, O L., *et al.*, 2009 *Cancer Sci.* 100, 2014–2021.
 Sanderson, J. P. *et al.*, 2020 *Oncoimmunology* 9, 1682381.

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.

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PRAME TCR-T Cells	82391	1 vial
MAGE-A4 TCR T Cells	82390	1 vial
Untransduced T Cells (MAGE-A4 TCR-T Negative Control)	82396	1 vial
IFN-γ (Human) Colorimetric ELISA Detection Kit	79771	96 reactions/ 5 x 96 reactions
IL-2 (Human) Colorimetric ELISA Detection Kit	79774	96 reactions/ 5 x 96 reactions
PRAME TCR CD8+ NFAT-Luciferase Reporter Jurkat Cell Line	78997	2 vials

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