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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
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Description

The MAGE-A4 TCR-T Cells are generated by high-titer lentiviral transduction of human primary CD4⁺ and CD8⁺ T cells with MAGE-A4-Specific TCR Lentivirus (#78935). These ready-to-use TCR (T cell receptor)-T cells express the human TCR that specifically recognizes antigen MAGE-A4 (Melanoma-associated antigen 4).

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

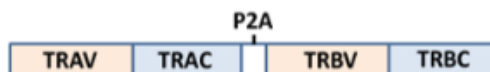


Figure 1: Construct diagram showing the expressed components of the MAGE-A4-specific TCR expressed in MAGE-A4 TCR-T Cells.

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

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 tend 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 (s)

- Positive control in MAGE-A4 TCR-T cell development.
- Screen modulators of MAGE-A4 TCR-T cell-driven cytotoxicity.
- Design and optimize co-culture bioassays for MAGE-A4-specific TCR-T cell evaluation.

Materials Provided

Components	Format
One vial of frozen cells	Each vial contains 5×10^6 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
T2 Cell Line	ATCC #CRL-1992
MAGE-A4 Peptide (230-239)	BPS Bioscience #78966
PRAME Peptide (425-433)	BPS Bioscience #78991
PE MHC I Dextramer (HLA-A*02:01 GVYDGREHTV)	Immudex #WB03578
Untransduced T Cells (MAGE-A4 TCR-T Negative Control)	BPS Bioscience #82396
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	MedchemExpress #HY-N0150
Brefeldin A	MedchemExpress #HY-16592
Cell Staining Buffer	BioLegend #420201
Fixation Buffer	BioLegend #420801
Permeabilization Wash Buffer	BioLegend #421002

Recommended TCR-T Cell Medium: TCellIM™ (#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% CO₂ 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.

- Continue to culture the cells at 37°C with 5% CO₂.
- 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 MAGE-A4 TCR-T cells are activated for expansion after thawing. Since these are primary cells that have been already cultured, the extent of expansion is not predictable. Perform the cytotoxicity assay as soon as possible to avoid T cell exhaustion. MAGE-A4 TCR-T Cells should not be in culture for more than 5 days. It is not recommended to freeze the cells again.

Validation

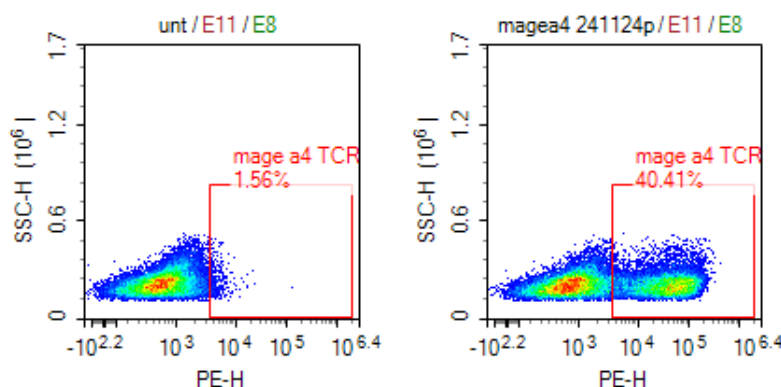


Figure 2: Expression of MAGE-A4 TCR in MAGE-A4 TCR-T Cells.

MAGE-A4 TCR-T cells (right) and Untransduced T cells (#82936) (left) were thawed and cultured for 24 hours. ~50,000 cells were stained with PE-labeled MHC I Dextramer (HLA-A*02:01 GVYDGREHTV) (Immudex #WB03578) and analyzed by flow cytometry. The y axis represents the side scatter height, while the x axis indicates PE-intensity.

Functional Validation

- The following experiments are examples of co-culture assays used to evaluate the cytotoxicity potential of MAGE-A4 TCR-T cells by **A)** Intracellular IFN- γ cytokine staining and **B)** Degranulation Assay.

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

- The assay described was performed in a 24-well plate with a 500 μ l 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 (#82936) as negative control.
 - We recommend including the following controls: “No T2 Cells”, and “PRAME Peptide” (as irrelevant peptide control).
- Thaw and culture MAGE-A4 TCR-T cells and Untransduced T Cells (#82936) for 24 hours in TCR-T Cell Medium according to the protocol in the “Cell Culture Protocol” section above.
 - Co-culture 5 x 10⁵ Untransduced T or MAGE-A4 TCR-T cells with 1 x 10⁵ T2 cells pulsed with 1 μ M of MAGE-A4 peptides, in TCR-T Cell Medium, for each well of the “Test Condition”.

3. Co-culture 5×10^5 Untransduced T or MAGE-A4 TCR-T cells with 1×10^5 T2 cells pulsed with 1 μ M of PRAME peptides, in TCR-T Cell Medium, for each well of the "PRAME Peptide" (irrelevant peptide control).
4. Keep 5×10^5 Untransduced T or MAGE-A4 TCR-T cells without T2 cells, as "No T2 Cells" condition.
5. Incubate the cells at 37°C in 5% CO₂ for 6 hours.

Optional: Add 2 μ M of Monensin and 3 μ M of Brefeldin A to each well 1 hour after co-culture, to aid with intracellular cytokine staining, and incubate the cells at 37°C in 5% CO₂ for another 5 hours.

6. Wash cells twice with Cell Staining Buffer and stain with Anti-CD8 Antibody, FITC-Labeled for 30 minutes at Room Temperature (RT).
7. Wash cells twice with Cell Staining Buffer and fix for intracellular staining using the Fixation/Permeabilization Buffers according to the manufacturer's instructions.
8. Analyze cells by flow cytometry.

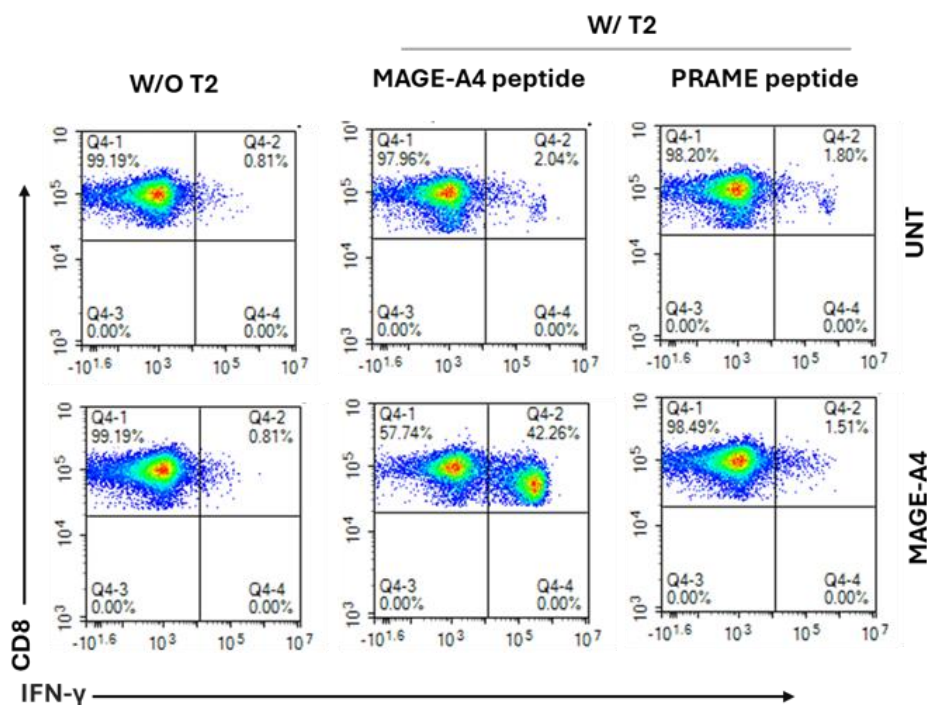


Figure 3: Flow cytometry analysis of IFN- γ cytokine production in MAGE-A4 TCR-T Cells co-cultured with T2 cells pulsed with different peptides.

MAGE-A4 TCR-T cells (MAGE-A4) and Untransduced T cells (UNT) were co-cultured either with MAGE-A4 antigen-pulsed T2 cells or with antigen-negative (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-Labeled (BPS Bioscience #102224) and intracellularly with APC anti-human IFN- γ (BioLegend #986702) and analyzed by flow cytometry. The x axis represents APC intensity, while the y axis shows FITC intensity.

B. Degranulation Assay

- The assay described was performed in a 96-well plate with a 100 μ l 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 (#82936) as negative control.
 - We recommend including the following controls: “No T2 Cells” and “PRAME Peptide” (as irrelevant peptide control).
- Thaw and culture MAGE-A4 TCR-T cells and Untransduced T Cells for 24 hours in TCR-T Cell Medium according to the protocol in the “Cell Culture Protocol” section above.
 - Co-culture 1×10^5 Untransduced T or MAGE-A4 TCR-T cells with 2×10^4 T2 cells pulsed with 1 μ M of MAGE-A4 peptides, in TCR-T Cell Medium, for the each well of the “Test Condition”, with 5 μ l of APC anti-human CD107a (LAMP-1) Antibody.
 - Co-culture 1×10^5 Untransduced T or MAGE-A4 TCR-T cells with 2×10^4 T2 cells pulsed with 1 μ M of PRAME peptides, in TCR-T Cell Medium, for each well of the “PRAME Peptide” (irrelevant peptide control), with 5 μ l of APC-labeled anti-human CD107a (LAMP-1) Antibody.
 - Keep 1×10^5 Untransduced T or MAGE-A4 TCR-T cells without T2 cells, as “No T2 Cells” condition, with 5 μ l of APC-labeled anti-human CD107a (LAMP-1) Antibody.
 - Incubate the cells at 37°C in 5% CO₂ for 6 hours.

Optional: Add 2 μ M of Monensin and 3 μ M of Brefeldin A to each well 1 hour after co-culture, to aid with surface CD107a staining, and incubate the cells at 37°C in 5% CO₂ for another 5 hours.

- Wash cells twice with Cell Staining Buffer and stain with Anti-CD8 Antibody, FITC-Labeled for 30 minutes at RT.
- Analyze cells by flow cytometry.

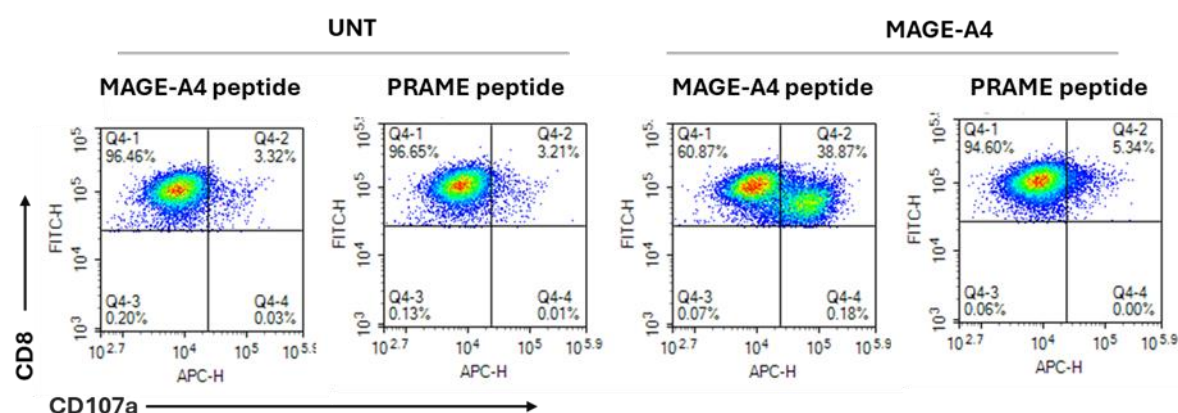


Figure 4: Flow cytometry analysis of CD107a degranulation in MAGE-A4 TCR-T Cells co-cultured with T2 cells pulsed with different peptides.

MAGE-A4 TCR-T cells (MAGE-A4) and Untransduced T cells (UNT) were co-cultured either with MAGE-A4 antigen-pulsed T2 cells or with antigen-negative (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-Labeled (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

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.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PRAME TCR T Cells	82391	1 vial
Untransduced T Cells (PRAME TCR-T Negative Control)	82397	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
MAGE-A4 CD8+ NFAT-Luciferase Reporter Jurkat Cell Line	78984	2 vials

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