Description

The PRAME TCR-T Cells are generated by high-titer lentiviral transduction of human primary $CD4^+$ and $CD8^+$ T cells with PRAME-Specific TCR Lentivirus (#78959). These ready-to-use TCR (T cell receptor)-T cells express the human TCR that specifically recognizes antigen PRAME (Preferentially Expressed Antigen in Melanoma).

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

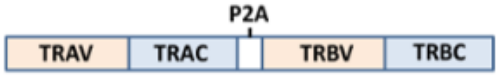

Figure 1: Construct diagram showing the expressed components of the PRAME-specific TCR expressed in PRAME TCR-T Cells.

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

PRAME (Preferentially Expressed Antigen in Melanoma) is a protein with a profile of expression in normal tissues highly restricted to testis, ovarium, and endometrium. However, it is found at high levels in several cancer types, such as melanoma, breast, and lung cancer. It is also found in cells of patients with AML (acute myeloid leukemia) and Hodgkin's lymphoma. Overexpression seems to block retinoic acid-mediated cell proliferation, differentiation, and apoptosis, contributing to tumorigenesis. Its expression pattern makes it an attractive target for immunotherapy. It is a membrane-bound protein, and it is thus a good target for TCR (T cell receptor)-T cells and anti-PRAME vaccines. Several clinical trials are ongoing and have demonstrated the clinical potential of targeting PRAME in melanoma, lung cancer and other solid tumors. Further studies into the functions of this protein will bring new clinical advances in cancer therapy.

Application (s)

- Positive control in PRAME TCR-T cell development.
- Screen modulators of PRAME TCR-T cell-driven cytotoxicity.
- Design and optimize co-culture bioassays for PRAME-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
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 (PRAME TCR-T Negative Control)	BPS Bioscience #82397
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: 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, at a minimum of 0.5 x 10^6 cells/ml.

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

Validation

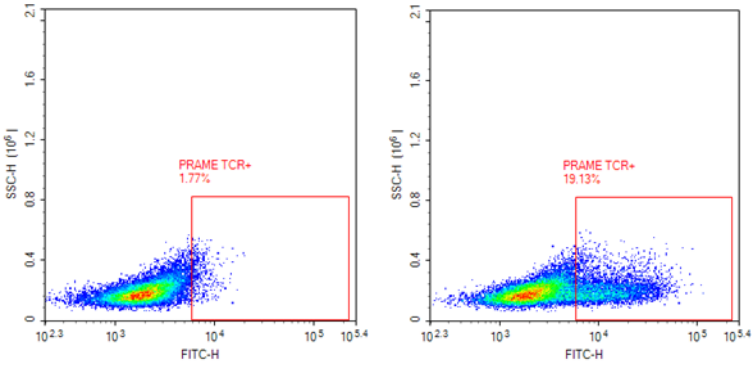

Figure 2: Expression of PRAME TCR in PRAME TCR-T Cells assessed by flow cytometry.
PRAME TCR-T cells (right) and Untransduced T cells (#82937) (left) were thawed and cultured for 24 hours. ~50,000 cells were stained with FITC-labeled MHC I Dextramer (HLA-A*02:01 SLLQHLIGL) (Immudex #WB04074) and analyzed by flow cytometry. The y axis represents the side scatter height, while the x axis indicates FITC-intensity.

Functional Validation

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

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

- The 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 (#82937) as negative control.
- We recommend including the following controls: “No T2 Cells”, and “MAGE-A4 Peptide” (as irrelevant peptide control).

1. Thaw and culture PRAME 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.

2. Co-culture 5 x 10^5 Untransduced T or PRAME TCR-T cells with 1 x 10^5 T2 cells pulsed with 1 µM of PRAME peptides, in TCR-T Cell Medium, for each well of the “Test Condition”.

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

4. Keep 5 x 10^5 Untransduced T or PRAME TCR-T cells without T2 cells, as "No T2 Cells" condition.

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

   Optional: Add 2 µM of Monensin and 3 µM 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% CO_2 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.

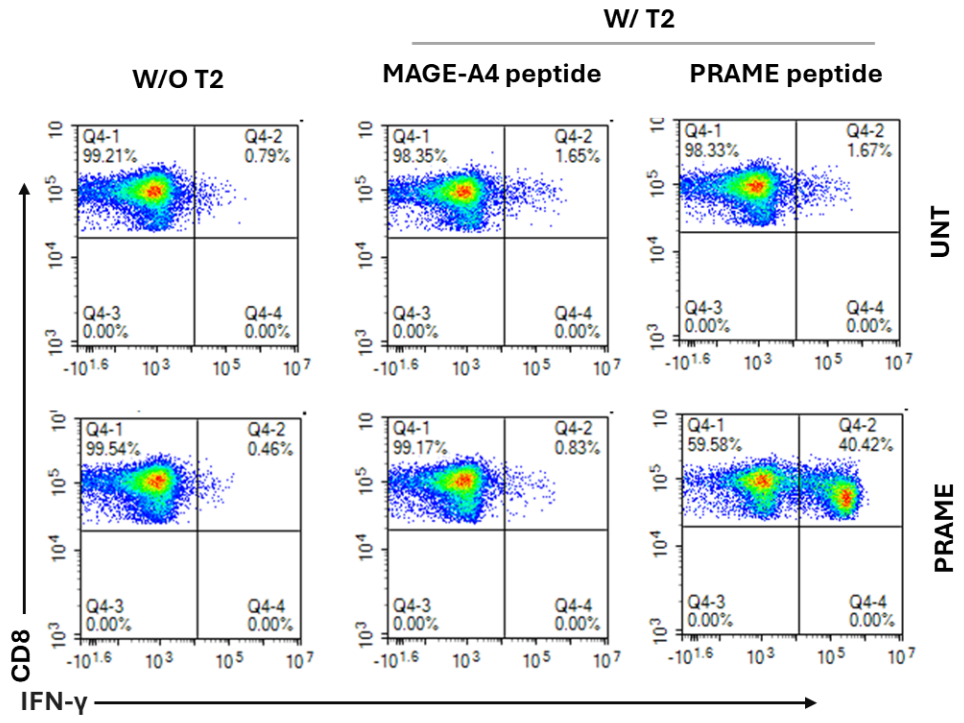

Figure 3: Flow cytometry analysis of IFN-γ cytokine production in PRAME TCR-T Cells co-cultured with T2 cells pulsed with different peptides.
PRAME TCR-T cells (PRAME) and Untransduced T cells (UNT) were co-cultured either with PRAME antigen-pulsed T2 cells or with antigen-negative (irrelevant peptide) 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

- 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 (#82937) as negative control.
- We recommend including the following controls: "No T2 Cells" and "MAGE-A4 Peptide" (as irrelevant peptide control).

1. Thaw and culture PRAME 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.

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

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

4. Keep 1 x 10^5 Untransduced T or PRAME TCR-T cells without T2 cells, as "No T2 Cells" condition, with 5 µl of APC-labeled anti-human CD107a (LAMP-1) Antibody.

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

   Optional: Add 2 µM of Monensin and 3 µM 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% CO_2 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. Analyze cells by flow cytometry.

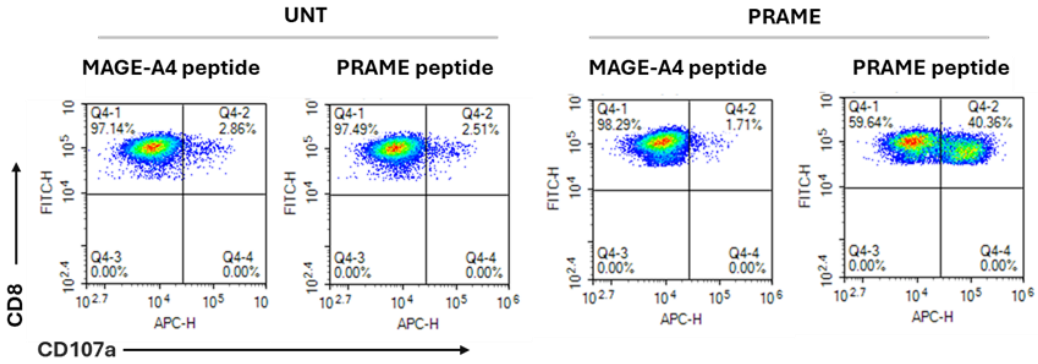

Figure 4: Flow cytometry analysis of CD107a degranulation in PRAME TCR-T Cells co-cultured with T2 cells pulsed with different peptides.

PRAME TCR-T cells (PRAME) and Untransduced T cells (UNT) were either co-cultured with PRAME 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 K.N., *et al.*, 2020 *PLOS One* 15(9): e0238875.
Salmaninejad A., *et al.*, 2016 *Immunol Invest* 45(7):619-40.

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*
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
MAGE-A4 CD8+ NFAT-Luciferase Reporter Jurkat Cell Line	78984	2 vials

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