**Description**

The Dual Epitope Anti-BCMA CAR-T Cells are generated via high-titer lentiviral transduction of human primary $CD4^+$ and $CD8^+$ T cells with the SIN Anti-BCMA CAR Lentivirus (VHH1/VHH2 ScFv-CD8-4-1BB-CD3ζ) (#78783). These ready-to use CAR (chimeric antigen receptor)-T cells express an anti-BCMA CAR consisting of a ScFv (single-chain variable fragment) that recognizes two BCMA epitopes (VHH1 and VHH2) linked to a CD8 hinge and transmembrane domains, and the 4-1BB and CD3ζ signaling domains (Figure 1).

These CAR-T cells have been validated by flow cytometry (to determine the CAR expression) and in co-culture cytotoxicity assays.


*Figure 1: Construct diagram showing components of the anti-BCMA CAR expressed in Dual Epitope Anti-BCMA CAR-T Cells.*

**Background**

BCMA (B-cell maturation antigen), also known as CD269 or tumor necrosis factor receptor superfamily member 17 (TNFRSF17), is a cell surface receptor of the TNF (tumor necrosis factor) receptor superfamily that recognizes BAFF (B-cell activating factor). BCMA is preferentially expressed in mature B lymphocytes and in Multiple Myeloma (MM) cells. BCMA is a highly attractive target antigen for immunotherapy because of its restricted expression in nonmalignant tissue but almost universal expression on MM cells. BCMA CAR-Ts are primary T cells engineered by lentiviral transduction to express a fully human-specific BCMA-CAR. CAR-T cells targeting BCMA have shown clinical anti-MM activity, and in 2022, the FDA granted authorization to use LCAR-B38M CAR-T Cell immunotherapy in MM. Active research into the development of BCMA targeting CARs for the treatment of several oncogenic disorders is ongoing, and represents a promising therapeutic avenue in cancer therapy.

**Application(s)**

- Positive control in anti-BCMA CAR-T development.
- Screen modulators of anti-BCMA CAR-T driven cytotoxicity.
- Design and optimize co-culture cytotoxicity assays.

**Biosafety**

The Dual Epitope Anti-BCMA CAR-T Cells are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle.

**Materials Provided**

| Components | Format |
|---|---|
| One vial of frozen cells | Each vial contains >1 x $10^6$ cells in 1 ml of CryoStor® CS10 (Stemcell Technologies #07942) |

**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 CAR-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 |
| BCMA, Fc-Fusion, Avi-Tag, PE-Labeled Recombinant | BPS Bioscience #100733 |
| Untransduced T Cells (as Negative Control for CAR-T cells) | BPS Bioscience #78170 |
| Firefly Luciferase CHO Cell Line | BPS Bioscience #79725 |
| BCMA/Firefly Luciferase CHO Cell Line | BPS Bioscience #79724 |
| Firefly Luciferase RPMI8226 Cell Line | BPS Bioscience #79834 |
| Thaw Medium 3 | BPS Bioscience #60186 |
| Thaw Medium 10 | BPS Bioscience #79704 |
| ONE-Step™ Luciferase Assay System | BPS Bioscience #60690 |
| Luminometer | |

**Recommended Anti-BCMA CAR-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 Anti-BCMA CAR-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 Anti-BCMA CAR-T Cell Medium.

3. Transfer the resuspended cells to a T25 flask.

4. If desired, activate the cells using Human CD3/CD28/CD2 T Cell Activator, following the manufacturer's recommendations, 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 Anti-BCMA CAR-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 2.0 x $10^6$ cells/ml. Transfer the cells into larger culture vessels and add fresh medium when the density reaches 2.0 x $10^6$ cells/ml.

Perform the cytotoxicity assay as soon as possible to avoid T cell exhaustion. Dual Epitope Anti-BCMA CAR-T Cells may stop proliferating after ~one week in culture. Cells can be activated again for expansion. It is not recommended to freeze the cells again once they have been activated and expanded.

**Validation**

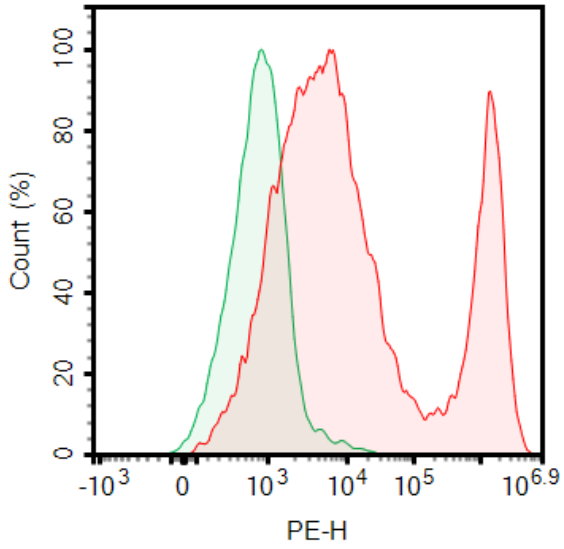


*Figure 2: Expression of anti-BCMA CAR in Dual Epitope Anti-BCMA CAR-T Cells.*
Dual Epitope Anti-BCMA CAR-T cells (red) were thawed and cultured for 24 hours. ~50,000 cells were stained with 5 µg/ml of BCMA, Fc-Fusion, Avi-Tag, PE-Labeled Recombinant (#100733) in a final volume of 50 µl and analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates PE-intensity.

**Functional Validation**

- The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include “Luminescence Background”, “No T Cell Control” (contain Luciferase CHO cells but no T cells) and “Test Condition” wells.
- The following experiments are two examples of co-culture assays used to evaluate the cytotoxicity of Dual Epitope Anti-BCMA CAR-T Cells against **A)** BCMA/Firefly Luciferase CHO Cell Line or **B)** Firefly Luciferase RPMI 8226 Cell Line as the target cells.

- We recommend using Untransduced T Cells as negative control.
- We recommend using Firefly Luciferase CHO Cell Line as control also for assay A.

**A. Cytotoxicity assay using BCMA/Firefly Luciferase CHO Cell Line as target**

**Day 1:**

1. Thaw Dual Epitope Anti-BCMA CAR-T cells and Untransduced T Cells, activate, and expand according to the protocol described in the "**Cell Culture Protocol"** section.

2. Seed BCMA/Firefly Luciferase CHO cells and negative control Firefly Luciferase CHO cells at 500 cells/well in 50 µl of Thaw Medium 3 in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Luminescence Background" wells.

3. Centrifuge Dual Epitope Anti-BCMA CAR-T cells and Untransduced T cells at 300 *x g* for 5 minutes and resuspended the cell pellet in fresh Anti-BCMA CAR-T Cell Medium.

4. Determine the desired Effector to Target ratio (E:T) and prepare appropriate cell suspensions (50 µl/well).

5. Carefully pipet 50 µl of T cell suspension into the appropriate "Test Condition" wells, already containing the BCMA/Firefly Luciferase CHO or Firefly Luciferase CHO cells.

6. Add 50 µl of Anti-BCMA CAR-T Cell Medium to the "No T Cell Control" wells already containing the BCMA/Firefly Luciferase CHO or Firefly Luciferase CHO cells.

7. Add 100 µl of Anti-BCMA CAR-T Cell Medium to the "Background Luminescence" wells.

8. Incubate the plates at 37°C with 5% $CO_2$ for 24 hours.

   *Note: No overnight attachment is needed for CHO cell culture. T cells can be added into the wells right after the CHO cells were seeded.*

**Day 2:**

1. Pipet each well gently up and down 3 to 4 times.

2. Remove the medium containing the non-attached cells to another plate (100 µl).

3. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.

4. Incubate at room temperature for ~15 to 30 minutes.

5. Measure luminescence using a luminometer.

**Data Analysis**: the average background luminescence was subtracted from the luminescence reading of all wells. The luciferase activity of Firefly Luciferase CHO cells or BCMA/Luciferase CHO cells alone, no T cells present, was set as 100%. The % Luminescence was calculated as: (Luminescence from the co-culture well)/(Luminescence from the "No T Cell Control" well).

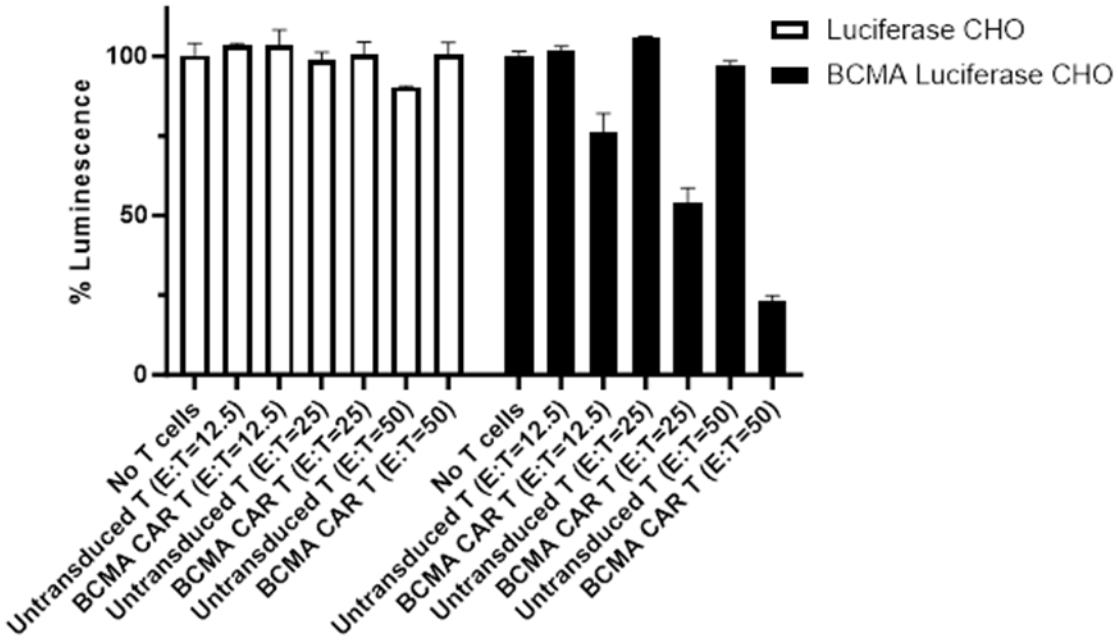


*Figure 3: Luciferase-based cytotoxicity assay using Dual Epitope Anti-BMCA CAR-T Cells co-cultured with BCMA-Luciferase CHO cells as target cells.*
Dual Epitope Anti-BCMA CAR-T cells and Untransduced T cells were thawed and expanded for 24 hours. The Dual Epitope Anti-BCMA CAR-T cells (effector cells) were then co-cultured with BCMA/Luciferase CHO cells (target) for 24 hours at the indicated effector:target (E:T) ratio. Luciferase activity was measured using ONE-Step™ Luciferase assay reagent. Dual Epitope Anti-BCMA CAR-T cells showed specific toxicity towards BCMA/Luciferase CHO cells. Untransduced T cells (#78170) and Firefly Luciferase CHO cells (#79725) were run in parallel as negative controls.

**B. Cytotoxicity assay using Firefly Luciferase - RPMI 8226 Recombinant Cell Line as target**

1. Thaw T cells, activate, and expand according to the protocol described in the "**Cell Culture Protocol"** section.

2. Seed Firefly Luciferase - RPMI8226 cells, which express endogenous BCMA, at 5,000 cells/well in 50 µl of Thaw Medium 10 in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Luminescence Background" wells.

3. Centrifuge T cells at 300 *x g* for 5 min and resuspended the cell pellet in fresh Anti-BCMA CAR-T Cell Medium.

4. Determine the desired Effector to Target ratio (E:T) and prepare appropriate cell suspensions (50 µl/well).

5. Carefully pipet 50 µl of T cell suspension into the appropriate "Test Condition" wells, containing the Firefly Luciferase RPMI 8226 cells.

6. Add 50 µl of Anti-BCMA CAR-T Cell Medium to the "No T Cell Control" wells.

7. Add 100 µl of Anti-BCMA CAR-T Cell Medium to the "Background Luminescence" wells.

8. Incubate the plates at 37°C with 5% $CO_2$ for 24 hours.

**Day 2:**

1. Pipet each well gently up and down 3 to 4 times.
2. Transfer the medium containing the non-attached cells to another plate (100 µl).
3. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.
4. Incubate at room temperature for ~15 to 30 minutes.

**Data Analysis:** the average background luminescence was subtracted from the luminescence reading of all wells. The luciferase activity of Firefly Luciferase- RPMI 8226 Recombinant Cell Line was set as 100%. The % Luminescence was calculated as: (luminescence of co-culture well)/ (luminescence from the "No T Cell Control" well).

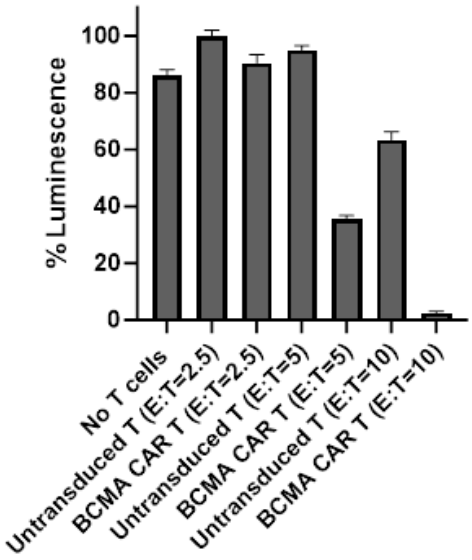


*Figure 4: Luciferase-based cytotoxicity assay using Dual Epitope Anti-BMCA CAR-T Cells co-cultured with Firefly Luciferase - RPMI 8226 Recombinant Cell Line as the target cells.*
Dual Epitope Anti-BCMA CAR-T cells and control Untransduced T cells (#78170) were thawed and expanded for 4 days. T cells (effector cells) were then co-cultured with Firefly Luciferase - RPMI 8226 cells for 24 hours at the indicated effector:target ratio (E:T). Luciferase activity was measured using ONE-Step™ Luciferase assay reagent.

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

**References**

Ghosh A., *et al.*, 2017 *Leuk Lymphoma.* 6:1-12
Sanchez E., *et al.*, 2018 *Expert Rev Mol Diagn.* 7:1-11.
Sohail A., *et al.*, 2018 *Immunotherapy.* 10(4):265-282.
Sidaway P., *et al.*, 2016 *Nat Rev Clin Oncol.* 13(9):530.

**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* |
|---|---|---|
| Anti-BCMA CAR Lentivirus (Clone C11D5.3 ScFv-CD8-4-1BB-CD3ζ) | 78655 | 50 µl |
| BCMA CRISPR/Cas9 Lentivirus (Integrating) | 78893 | 500 µl x 2 |
| BCMA CRISPR/Cas9 Lentivirus (Non-Integrating) | 78894 | 500 µl x 2 |
| Anti-BCMA-Anti-CD3 Bispecific Molecule | 100689 | 50 µg/100 µg |
| BCMA CHO Recombinant Cell Line (High or Low Expression) | 79500 | 2 vials |
| Anti-BCMA Antibody | 101219 | 100 µg |

*Version 032724*