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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Anti-CD19 CAR-Gamma Delta T Cells (δ2) are produced by high-titer lentiviral transduction of human peripheral blood gamma delta 2 (δ2) T cells using the Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector, #78601). These ready-to use CAR (chimeric antigen receptor)-Gamma Delta T cells express an anti-CD19 CAR consisting of the ScFv (Single chain fragment variable) of anti-CD19 (clone FMC63) linked to a 2nd generation CAR containing the CD8 hinge and transmembrane domains, and the 4-1BB and CD3ζ signaling domains (Figure 1).

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

Figure 1: Construct diagram showing components of the anti-CD19 CAR expressed in Anti-CD19 CAR- Gamma Delta T Cells (δ2).

Background

T lymphocytes are composed of two subpopulations: αβ T cells and γδ T cells. They are distinguished by the expression of either a TCR or a γδ TCR, respectively. αβ T cells are the predominant subset of T cells in peripheral blood and recognize antigens presented by MHC (major histocompatibility complex) molecules. γδ T cells are less abundant and recognize antigens independently of MHC presentation. While both αβ T cells and γδ T cells contribute to cell cytotoxicity through distinct mechanisms to target and eliminate infected or abnormal cells, γδ T cells have a lower risk of causing GvHD (Graft-versus-Host Disease) when injected into humans and have demonstrated cytotoxicity against a wide range of tumor types. γδ TCRs are cell type specific, with Vγ9Vδ2 being present in T cells and corresponding to about 5% of the T cell population in blood. Vγ9Vδ2 T cells are involved mostly in immune responses to pathogens and long-term modulation of inflammation, and can recognize non-peptide phosphor-antigens, alkylamines and synthetic amino-bisphosphonates. Vγ9Vδ2 T cells are being studied for the treatment of solid tumors and hematological disorders and are becoming a highly promising cancer therapy. Further studies on how best to utilize Vγ9Vδ2 T cells, and methods to enhance their presence, will open new therapeutic avenues for cancer and infections.

Application

- Use as positive control in the development of anti-CD19 CAR- Gamma Delta T Cells (δ2).
- Screen modulators of anti-CD19 CAR-Gamma Delta T Cell (δ2) cytotoxicity.
- Design and optimize co-culture cytotoxicity assays for anti-CD19 CAR-Gamma Delta T cell (δ2) specific evaluation.

Materials Provided

Components	Format
1 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cryopreservation solution

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.

Materials Required but Not Supplied

These materials are not supplied but are necessary for cell culture and for the cellular assays described below. BPS Bioscience's reagents are validated and optimized for use with this expansion kit and are highly recommended for the best results.

Name	Ordering Information
TCellM™	BPS Bioscience #78753
Human Interleukin-2 Recombinant	BPS Bioscience #90184
Membrane Bound IL-15 Based Growth-Arrested Feeder Cells	BPS Bioscience #82374
PE anti-human TCR Vδ2 Antibody	BioLegend #331408
PE-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1 (FM3-HPY53)	ACROBiosystems #FM3-HPY53-25tests
Untransduced Gamma Delta T Cells (δ2)	BPS Bioscience #82498
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
Firefly Luciferase CD19 Knockout Raji Cell Line	BPS Bioscience #82167
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2D	BPS Bioscience #79639
Growth Medium 2E	BPS Bioscience #79638
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Recommended Gamma Delta T Cells Medium: TCellM™ (#78753) supplemented with 50 ng/ml Interleukin-2 (#90184).

Cell Culture Protocol

Note: Anti-CD19 CAR-Gamma Delta T cells (δ2) are derived from human material and thus the use of adequate safety precautions is recommended.

CAR-Gamma Delta T 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 Gamma Delta T Cells 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 Gamma Delta T Cells Medium.

3. Transfer the resuspended cells to a T25 flask.

4. If desired, co-culture the cells with Membrane Bound IL-15 Based Growth-Arrested Feeder Cells (#82374) at a 2:1 ratio of feeder cells: Anti-CD19 CAR-Gamma Delta T cells as described in the next steps.

Membrane Bound IL-15 Based Growth-Arrested Feeder Cell Thawing

1. Swirl the vial of frozen feeder cells (2 million/vial) 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 Thaw Medium 2.

   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 1 ml of pre-warmed Gamma Delta T Cells Medium.

CAR-Gamma Delta T Cell Culture

1. Centrifuge the cells gently at 300 *x g* for 5 minutes.

2. Resuspend cells in fresh Gamma Delta T Cells Medium.

3. Continue to culture the cells at 37°C with 5% CO_2.

4. Determine cell density every 2-3 days. When the cell density reaches 2 million/ml, dilute the cells to 0.25-1.0 x 10^6 cells/ml with Gamma Delta T Cells Medium.

5. Refresh medium every 2-3 days and refresh feeder cells by providing Anti-CD19 CAR-Gamma Delta T cells with a 2:1 ratio of feeder cells: Anti-CD19 CAR-Gamma Delta T cells weekly.

It is recommended to co-culture Gamma Delta T cells for expansion using Membrane Bound IL-15 Based Growth-Arrested Feeder Cells after thawing. The extent of expansion is donor dependent. Perform the cytotoxicity assay as soon as possible to avoid T cell exhaustion. The Gamma Delta T cells should not be in culture for more than 7 days. It is not recommended to freeze the cells again once they have been activated and expanded.

Validation Data

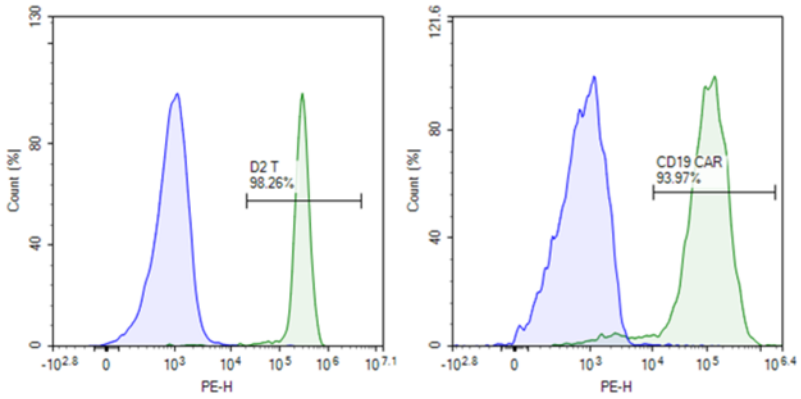

Figure 2: Expression of anti-CD19 CAR in anti-CD19 CAR-Gamma Delta T Cells (δ2) by flow cytometry.
Anti-CD19 CAR- Gamma Delta T cells (δ2) (green) and Untransduced Gamma Delta T cells (#82946) (blue) were thawed for 48 hours. Approximately 50,000 cells were analyzed by flow cytometry using PE anti-human TCR Vδ2 Antibody (BioLegend #331408) (left panel) and PE-labeled anti-FMC63 ScFv (Acrobiosystems #FM3-HPY53-25tests) (right panel). The y axis represents the % of cells, while the x axis indicates the PE intensity.

A. Cytotoxicity assay using Firefly Luciferase K562 Cell Line as target cells

- The following experiment is an example of a co-culture assay used to evaluate the cytotoxicity of Anti-CD19 CAR-Gamma Delta T cells using Firefly Luciferase K562 Cell Line (#78621) as target cells.
- The assay should include "No T Cell Control", "Background Luminescence" and "Test" wells.
- We recommend using Firefly Luciferase CD19 Knockout Raji Cell Line (#82167) as control.

Day 1:

1. Thaw Anti-CD19 CAR-Gamma Delta T cells and expand according to the protocol in the "Cell Culture Protocol" Section above.

Day 2:

1. Seed target cells, such as Firefly Luciferase K562 cells (#78621), in 50 µl of Thaw Medium 2 (#60184) at 5,000 cells/well in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Background Luminescence" control.

2. Centrifuge Anti-CD19 CAR-Gamma Delta T cells (δ2) gently (300 *x g* for 5 minutes) and resuspend in Gamma Delta T Cells Medium.

3. Prepare Anti-CD19 CAR-Gamma Delta T cells (δ2) at the appropriate cell density to reach the desired effector:target (E:T) cell ratio (50 µl/ well).

4. Pipet 50 µl of Anti-CD19 CAR-Gamma Delta T cells (δ2) into the "Test" wells, which contain target cells, at the desired effector:target (E:T) cell ratio.

5. Add 50 µl of Gamma Delta T Cells Medium to the "No T Cell Control" wells.

6. Add 100 µl of Gamma Delta T Cells Medium to the "Background Luminescence" wells.

7. Incubate the plates at 37°C with 5% CO_2 for 48 hours.

Day 3:

1. After 24 hours, add 100 µl of ONE-Step™ Luciferase assay reagent to each well and incubate at Room Temperature (RT) for ~15 to 30 minutes.

2. Measure luminescence using a luminometer.

B. Cytotoxicity assay using Firefly Luciferase Raji Cell Line as target cells

- The following experiment is an example of a co-culture assay designed to evaluate the cytotoxicity of Anti-CD19 CAR-Gamma Delta T Cells using Firefly Luciferase Raji Cell Line as the target cells.
- The assay should include "No T Cell Control" and "Background Luminescence" and "Test" wells.
- We recommend using Untraduced Gamma Delta T Cells (δ2) (#82498) as control.

Day 1:

1. Thaw Anti-CD19 CAR-Gamma Delta T cells and expand according to the protocol in the "Cell Culture Protocol" Section above.

Day 2:

1. Seed target cells, such as Firefly Luciferase Raji Cells (#78622) that endogenously express CD19, in 50 µl of Thaw Medium 2 (#60184) at 5,000 cells/well in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Background Luminescence" control.

2. Centrifuge Anti-CD19 CAR-Gamma Delta T cells (δ2) gently (300 *x g* for 5 minutes) and resuspend in Gamma Delta T Cells Medium.

3. Prepare Anti-CD19 CAR-Gamma Delta T cells (δ2) at the appropriate cell density to reach the desired effector:target (E:T) cell ratio (50 µl/well).

4. Pipet 50 µl of Anti-CD19 CAR-Gamma Delta T cells (δ2) into the "Test" wells, which contain target cells, at the desired effector:target (E:T) cell ratio.

5. Adde 50 µl of Gamma Delta T Cells Medium to the "No T Cell Control" wells.

6. Add 100 µl of Gamma Delta T Cells Medium to the "Background Luminescence" wells.

7. Incubate the plates at 37°C with 5% CO_2 for 48 hours.

Day 3:

1. After 24 hours, add 100 µl of ONE-Step™ Luciferase assay reagent to each well and incubate at RT for ~15 to 30 minutes.

2. Measure luminescence using a luminometer.

Data Analysis

The average background luminescence was subtracted from the luminescence reading of all wells. The Cytotoxicity Index was calculated as:
(background-subtracted luminescence of co-culture wells) / (background-subtracted luminescence of the "No Cell Control" wells).

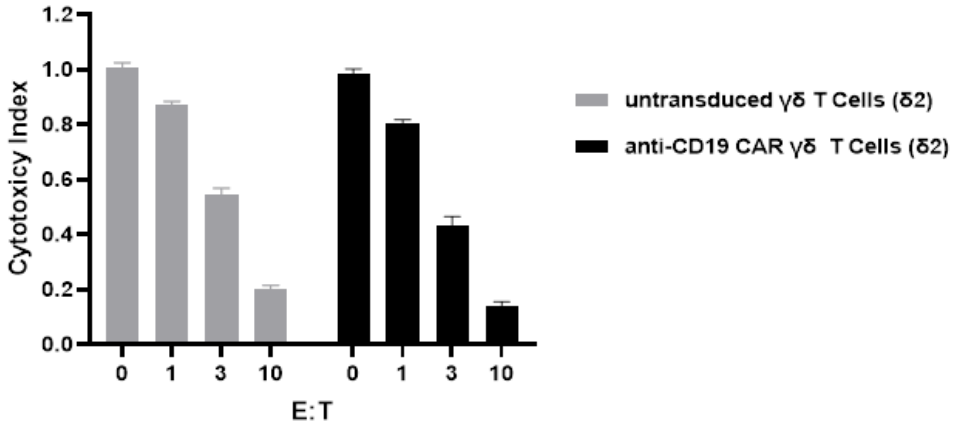

Figure 3: Luciferase-based innate cytotoxicity assay using Firefly Luciferase K562 Cell Line as target cells.

Anti-CD19 CAR-Gamma Delta T cells (δ2) and Untransduced Gamma Delta T cells (δ2) (#82498) were thawed 48 hours prior to the experiment. Anti-CD19 CAR-Gamma Delta T cells (δ2) (effector) were then co-cultured with Firefly Luciferase K562 cells for 24 hours at the indicated effector:target ratio. The lysis of target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase Assay System (#60690).

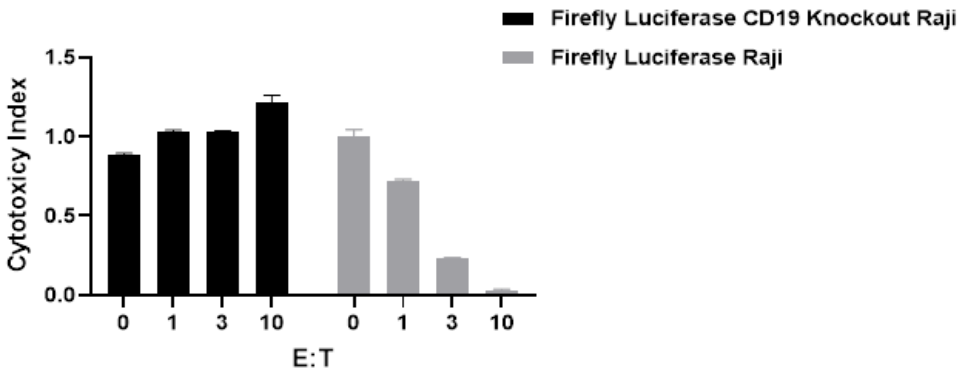

Figure 4: Luciferase-based CD19 specific cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells.

Anti-CD19 CAR-Gamma Delta T cells (δ2) were thawed 48 hours prior to the experiment. These cells (effector) were then co-cultured with Firefly Luciferase Raji cells and Firefly Luciferase CD19 Knockout Raji cells for 24 hours at the indicated effector:target ratios. The lysis of target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase Assay System (#60690).

Data shown is representative.

References

Allison T. and Garboczi D., 2002 *Molecular Immunology* 38 (14): 1051-1061.
Sawaisorn P., *et al.*, 2024 *Scientific Reports* 14: 1291.

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 precautions should be used.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions.

Related Products

Products	*Catalog #*	*Size*
Untransduced Gamma Delta T Cells (δ1)	82496	1 vial
Untransduced Gamma Delta T Cells (δ2)	82498	1 vial
Anti-CD19 CAR-Gamma Delta T Cells (δ1)	82497	1 vial
Vγ4Vδ1 TCR NFAT-Luciferase Reporter Jurkat Cell Line	82329	2 vials
Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line	82320	2 vials
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78556	2 vials

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