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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Untransduced Gamma Delta T Cells (δ2) are produced by mock lentiviral transduction of human peripheral blood gamma delta (δ2) T cells. These cells are subjected to comparable manipulations as CAR (chimeric antigen receptor)-Gamma Delta T cells (δ2) (example, #82499): activation, spinoculation (without lentivirus), and feeder expansion. These T cells are designed as negative controls in experiments using lentivirus-transduced 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 aminobisphosphonates. 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

- Negative control in experiments with lentivirus-transduced CAR-Gamma Delta T Cells (δ2).

Materials Provided

Components	Format
One vial 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. 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 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
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
Anti-CD19 CAR- Gamma Delta T Cells (δ2)	BPS Bioscience #82499
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2D	BPS Bioscience #79639
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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

Cell Culture Protocol

Note: Untransduced Gamma Delta T Cells (δ2) are derived from human material and thus the use of adequate safety precautions is recommended.

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 and co-culture with Membrane Bound IL-15 Based Growth-Arrested Feeder Cells (#82374) at 2:1 ratio of feeder cells: Gamma Delta T cells for up to 1 week if desired.

4. Centrifuge the cells gently at 300 *x g* for 5 minutes and resuspend in Gamma Delta T Cells Medium.

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

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

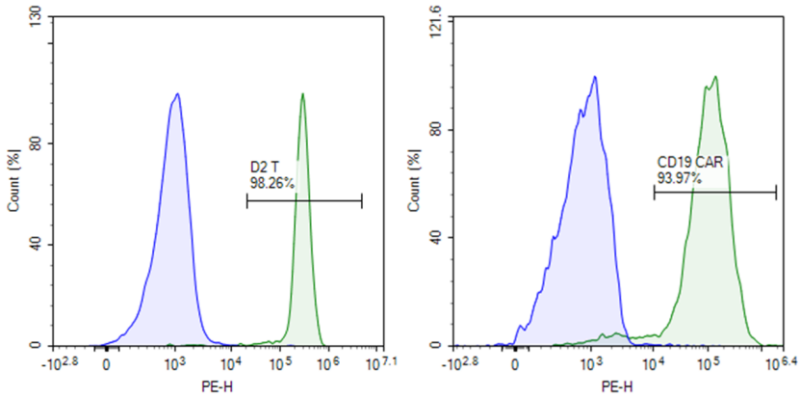

Figure 1: Expression of anti-CD19 CAR in anti-CD19 CAR- Gamma Delta T Cells (δ2) by flow cytometry.
Anti-CD19 CAR- Gamma Delta T cells (δ2) (#82499, green) and Untransduced Gamma Delta T cells (blue) were thawed for 48 hours. Approximately 50,000 cells were analyzed by flow cytometry using PE anti-human 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.

Cytotoxicity assay using Firefly Luciferase K562 Cell Line as the 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 as target cells.
- The assay should include “No T Cell Control”, “Background Luminescence” and “Test” wells.
- We recommend using Anti-CD19 CAR-Gamma Delta T cells (δ2) (#82499) as control.

Day 1:

1. Thaw Anti-CD19 CAR-Gamma Delta T cells and Untransduced 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 Gamma Delta T cells (gently (300 *x g* for 5 minutes) and resuspended in Gamma Delta T Cells Medium.

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

4. Pipet 50 µl of Untransduced Gamma Delta T cells (δ2) (#82498) or Anti-CD19 CAR- Gamma Delta T cells (δ2) (#82499) 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 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.

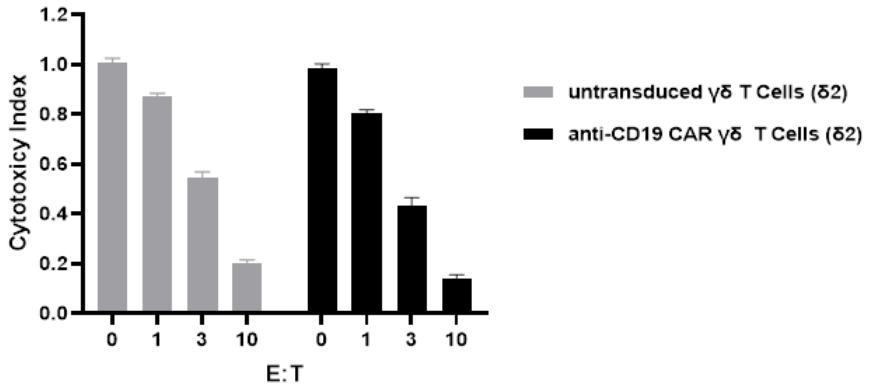

Figure 2: Luciferase-based cytotoxicity assay using Firefly Luciferase K562 Cell Line as target cells. Untransduced Gamma Delta T cells (δ2) (#82498) and Anti-CD19 CAR- Gamma Delta T cells (δ2) (#82499) were thawed 48 hours prior to the experiment. Gamma Delta T cells (effector) were then co-cultured 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).

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 Cell Line FAQs for more information. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Untransduced Gamma Delta T Cells (δ1)	82496	1 vial
Anti-CD19 CAR-Gamma Delta T Cells (δ1)	82497	1 vial
Anti-CD19 CAR-Gamma Delta T Cells (δ2)	82499	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

Version 050925