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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

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

Expanded Human Peripheral Blood Gamma Delta T Cells (Vδ1), Frozen, are primary Vδ1 T cells enriched and expanded from human PBMCs (Peripheral Blood Mononuclear Cells) using Membrane Bound IL-15 Based Growth-Arrested Feeder Cells (#82374), and cryopreserved. Expanded Human Peripheral Blood Gamma Delta T Cells (Vδ1), Frozen, are >90 % pure Vδ1 T cells, as measured by flow cytometry analysis. They can be used in cytotoxicity assays and other *in vitro* assays, after thawed in T Cell Medium (#78753), or further expanded if using feeder cells.

Background

T lymphocytes are composed of two subpopulations: αβ T-cells and γδ T-cells. They are distinguished by the expression of either an αβ 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, Vδ1 expressing T cells are found in mucosal and epithelial tissues and correspond to about 15% of the γδT cells present in PBMCs, with the % of γδ being only 5% of all the T cells. Vδ1 T cells are highly cytolytic, meaning they can effectively kill tumor cells. Engineering Vδ1 T cells to express chimeric antigen receptors (CARs) further enhances their antitumor activity and antigen specificity.

Application

Use in *in vitro* assays focused on immunological research, drug development, and cancer studies, including cytotoxicity assays, immunotherapy screening, drug screening, and immune activation assays.

Materials Provided

Components	Format
One vial of frozen cells	Each vial contains 2 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 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
FluoSite™ Anti-TCR γδ1 (gamma delta 1) Antibody, PE-Labeled	BPS Bioscience #102760
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
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	
Untransduced T Cells (Activated human αβ T Cells)	BPS Bioscience #78170

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

Cell Culture Protocol

Note: Gamma Delta T cells (Vδ1) are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing and Expansion

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 using 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 in 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. Since these are primary cells, the extent of expansion is not predictable. Perform the assays as soon as possible to avoid exhaustion. The Gamma Delta T cells should

not be in culture for more than 7 days without feeder cells. It is not recommended to freeze the cells again once they have been activated and expanded.

Validation

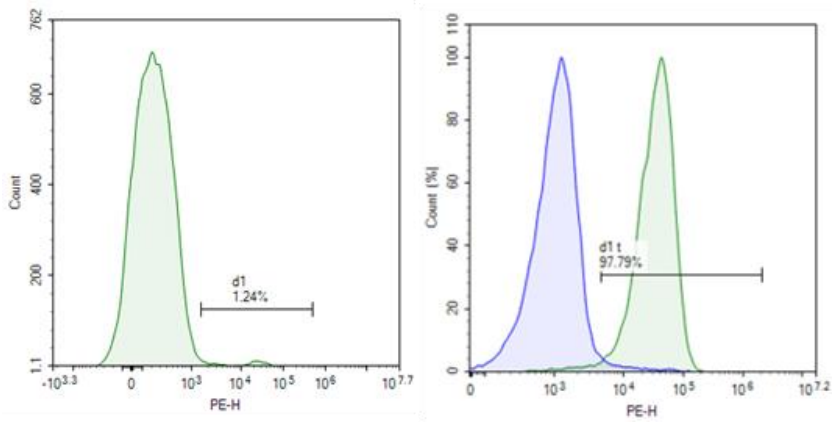

Figure 1: Vδ1 T cell marker assessment of the Expanded Human Peripheral Blood Gamma Delta T Cells (Vδ1), Frozen by flow cytometry.
Frozen Normal PBMCs (#79059) and 14-day expanded PBMC-derived Gamma Delta T cells (Vδ1) were thawed and stained with Fluosite™ Anti-TCR γδ1 (gamma delta 1) Antibody, PE-Labeled (#102760) and analyzed by flow cytometry. Representative flow cytometry plots show the percentage of Vδ1 T cells in the PBMCs (left) and expanded Gamma Delta T Cells (right, blue: Untransduced Gamma Delta T cells (δ2) (#82498) were used as negative control). 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 Expanded Human Peripheral Blood Gamma Delta T Cells (Vδ1), Frozen using Firefly Luciferase K562 Cell Line as the target cells.
- The assay should include “No T Cell Control”, “Background Luminescence” and “Test” wells.
- We recommend using Activated human αβ T Cells as negative control.

Day 1:

1. Thaw Expanded Human Peripheral Blood Gamma Delta T Cells (Vδ1) and culture cells according to the protocol described 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 Expanded Human Peripheral Blood T cells gently (300 *x g* for 5 minutes) and resuspended in Gamma Delta T Cells Medium.

3. Prepare Expanded Human Peripheral Blood 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 T cells 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 4:

1. After 24 hours, add 100 µl of ONE-Step™ Luciferase assay reagent to each well and incubate at Room Temperature 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 T Cell Control" wells).

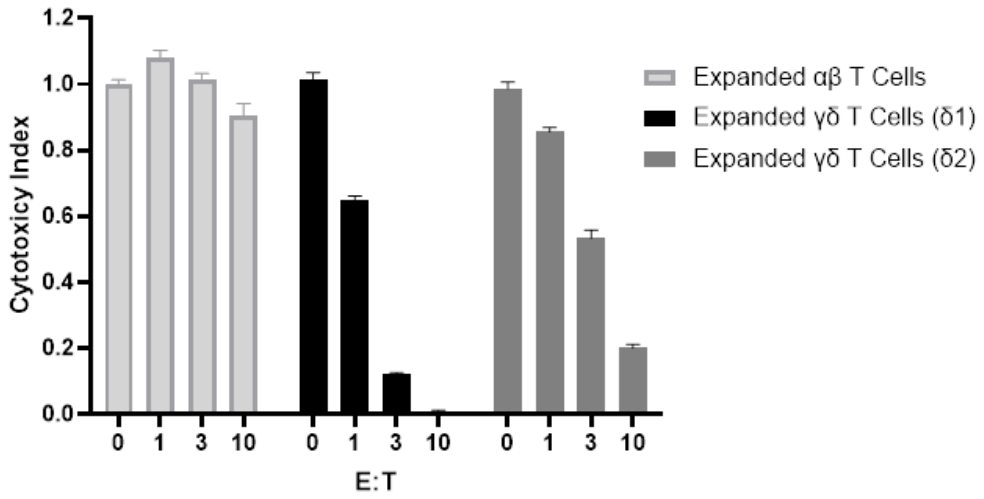

Figure 2: Luciferase-based cytotoxicity assay using Firefly Luciferase K562 Cell Line as the target cells.
Expanded Activated human αβ T cells and Gamma Delta T Cells were thawed 24 hours prior to the experiment. The Gamma Delta T cells (effector) were then co-cultured with Firefly Luciferase K562 cells 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.

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. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact

Related Products

Products	*Catalog #*	*Size*
Delta 1 (Vδ1) T Cell Expansion System	82456	1 kit
Vγ9Vδ2 T Cell Expansion Kit	82551	1 kit
Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2), Frozen	82733	1 vial
Untransduced Gamma Delta T Cells (δ2)	82498	1 vial
Anti-CD19 CAR-Gamma Delta T Cells (δ2)	82499	1 vial
Vγ4Vδ1 TCR Lentivirus	78986	100 µl; 500 µl x 2
Vγ9Vδ2 TCR Lentivirus	78985	100 µl; 500 µl x 2
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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