SZABO SCANDIC

Part of Europa Biosite

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

Delta 1 (Vδ1) T Cell Expansion System is suitable for the *ex vivo* culture and expansion of human Vδ1 (delta 1, D1) T cells. γδ T cells typically make up only 1-5% of total T cells in PBMCs (peripheral blood mononuclear cells) and therefore they need to be successfully activated and expanded to obtain an adequate number of cells for γδ T cell-based immunotherapy studies. This product contains media and reagents necessary to drive the robust activation and expansion of the Vδ1 T cells subpopulation from PBMCs. Vδ1 T Cell Expansion System is provided with enough reagents and materials for the *ex vivo* culture and expansion of human Vδ1 T cells from a starting population of 3 x 10^7 PBMCs. It is possible to use this kit for multiple expansions from smaller PBMC amounts.

Note: The use of this expansion system requires Anti-PE MicroBeads and MiniMACS™ Starting Kit (Miltenyi), that should be purchased separately (see information in Materials Required but not Supplied).

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, 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

Activation and expansion of Vδ1 T cell from freshly isolated for downstream applications in CAR (chimeric antigen receptor) γδ T cell development, cytotoxicity assays and flow cytometry.

Supplied Materials

Catalog #	Name	Amount	Storage
82374	Membrane Bound IL-15 Based Growth-Arrested Feeder Cells	2 million cells/vial x 10 vials	Liquid nitrogen
78753	TCellM™	500 ml	-20°C
90184	Human Interleukin-2	50 µg	-20°C
102760	Fluosite™Anti-TCRγδ1 (gamma delta 1) Antibody, PE-Labeled	30 µl	4°C

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. This assay kit will perform optimally for up to 6 months from the date of receipt when the materials are stored as directed.

Materials Required but Not Supplied

These materials are not supplied with the expansion kit but necessary for γδ T cell expansion and validation. BPS Bioscience's reagents are validated and optimized for use with this expansion kit and are highly recommended for the best results.

Materials Used in Cellular Assay

Name	Ordering Information
Normal Human Peripheral Blood Mononuclear Cells, Frozen	BPS Bioscience #79059
Anti-PE MicroBeads	Miltenyi Biotech #130-048-801
MiniMACS™ Starting Kit	Miltenyi Biotech# 130-090-312
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector)	BPS Bioscience #78601
RetroNectin® Recombinant Human Fibronectin Fragment	Takara #T100A
PE-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1 (FM3-HPY53)	Acrobiosystems #FM3-HPY53-25tests
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
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™ (BPS Bioscience #78753) supplemented with 50 ng/ml Interleukin-2 (BPS Bioscience #90184).

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Vδ1 T Cell Expansion Protocol

- The following protocol is a general guideline to expand Vδ1 T cells freshly isolated from PBMCs.
- The expansion fold obtained will vary, depending on the source of Vδ1 T cells and donor.
- The protocol may be adjusted at each step, but we recommend that cells do not reach over >2 million/ml.
- The following instructions are a general guideline for a starter cell number of 3 x 10^5 isolated Vδ1 T cells. If more cells are used as starting material, the volume of medium, amount of feeder cells, and culture vessels need to be scaled up accordingly.
- Flow cytometry analysis can be performed to monitor the Vδ1 T cells purity and expansion during the process.

Growth-Arrested Feeder Cells 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 content 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 1 ml of pre-warmed Gamma Delta T Cells Medium.

Vδ1 T Cell Expansion

1. Isolate Vδ1 T cells from 30 million of frozen PBMCs using MiniMACS™ system (Miltenyi Biotech# 130-090-312), following the manufacturer's user manual.

2. Combine isolated cells with:
   a. 20 µl of Fluosite™Anti-TCRγδ1 (gamma delta 1) Antibody, PE-Labeled (Vδ1 T cell activating antibody)
   b. 60 µl of Anti-PE MicroBeads(Miltenyi Biotech #130-048-801)

3. Culture isolated Vδ1 T cells (together with the bound antibody and microbeads) at 1 million/ml in Gamma Delta T Cells Medium for 48-hour (activation).

4. After 48 hours of activation, add feeder cells to Vδ1 T cells for expansion at a 2:1 ratio of feeder cells versus Vδ1 T cells.

   Note:
   a. For a starting culture, the total optimal cell number (Vδ1 T cells and feeder cells) is ~ 1.5 million/ml.
   b. Vδ1 T cells can also be engineering, for example by lentiviral transduction, after the 48-hour activation.
   c. The optimal feeder cells to Vδ1 T cells ratio may require optimization in your desired culture setup.

5. Grow the cells in a 5% CO_2 incubator at 37°C.

6. Determine cell density every other day and dilute the cell culture to 0.5-1 x 10^6 cells/ml with Gamma Delta T Cells Medium.

7. Refresh medium of Vδ1 T cells every 2-3 days and replenish culture with the same ratio (2:1) of feeder cells weekly.

Note: The protocol provided is a general guideline. Vδ1 T cell growth rates are donor dependent, and subculturing and feeding may need to be performed more frequently. We recommend monitoring culture density frequently and keeping it below 2 million/ml. Vδ1 T cells can be expanded for up to 3 weeks. Generally, a two-week expansion period can result in >1,000-fold expansion with > 90% cell purity.

CAR-Gamma Delta T Cell Engineering Protocol

The following protocol was used to transduce Vδ1 primary T cells with anti-CD19 CAR Lentiviruses. The transduction conditions (e.g. MOI, concentration of RetroNectin, time of assay development) should be optimized accordingly.

Day 1:

1. Isolate Vδ1 T cells from frozen human PBMC by following Vδ1 T Cell Expansion Protocol described above.
2. Resuspend Vδ1 T cells in Gamma Delta T Cells Medium at a density of 1 x 10^6 cells/ml.
3. Activate cells as described above for 48 hours.

Day 2:

1. Coat a non-treated, cell culture-grade 24-well plate at 4°C overnight with 500 µl of 20 - 100 µg/ml of RetroNectin, according to manufacturer's user manual.

Day 3:

1. Thaw CAR lentiviruses on ice.
2. Diluted the appropriate volume of lentiviruses in 500 µl of PBS to reach the desired MOI.
3. Add to the RetroNectin-coated plate.
4. Place the 24-well plate in a pre-warmed centrifuge to 32°C and centrifuge for 2 hours at 1,000 *x g*, to facilitate binding of virus particles to the RetroNectin reagent.
5. Discard the supernatant, but do not allow the plate to dry.
6. Wash the plate once with 500 µl of PBS.
7. Centrifuge the Vδ1 T cells and resuspend in fresh Gamma Delta T Cells Medium at 1 x 10^6 cells/ml.
8. Add 500 µl of cell suspension to each well of the coated 24-well plate.
9. Incubate at 37°C with 5% CO_2 for 48 hours.

Day 5-13:

1. Add fresh medium and feeder cells to expand CAR-Gamma Delta T cells.
2. Refresh medium every 2-3 days.
3. Refresh feeder cells by providing CAR-Gamma Delta T cells with a 2:1 ratio of feeder cells to CAR-Gamma Delta T cells weekly.

Day 13:

1. Analyze transduction efficiency by flow cytometry. For example, enriched CAR-Gamma Delta cells can be stained with PE- Monoclonal Anti-FMC63 Antibody (Acrobiosystems #FM3-HPY53-25tests) and Anti-PE MicroBeads (Miltenyi Biotech #130-048-801)

2. Expand CAR-Gamma Delta T cells for another week.

Day 20:

1. Freeze the CAR-Gamma Delta T cells or immediately use in downstream applications.

Validation Data

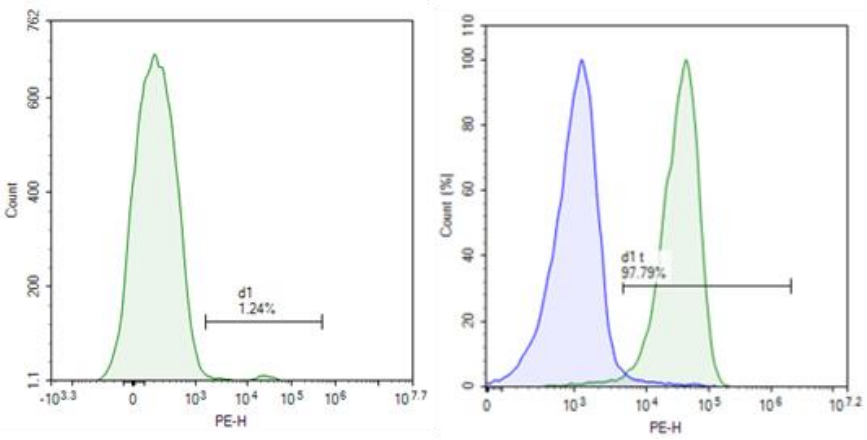

Figure 1: Vδ1 T cell marker assessment of expanded and frozen human Gamma Delta T cells Vδ1 by flow cytometry.
Frozen Normal PBMCs (#79059) and PBMC-derived Gamma Delta T cells (Vδ1) were expanded for 14 days and frozen, thawed and immediately stained with Fluosite™Anti-TCRγδ1 (gamma delta 1) Antibody, 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). The y axis represents the % of cells, while the x axis indicates the PE intensity.

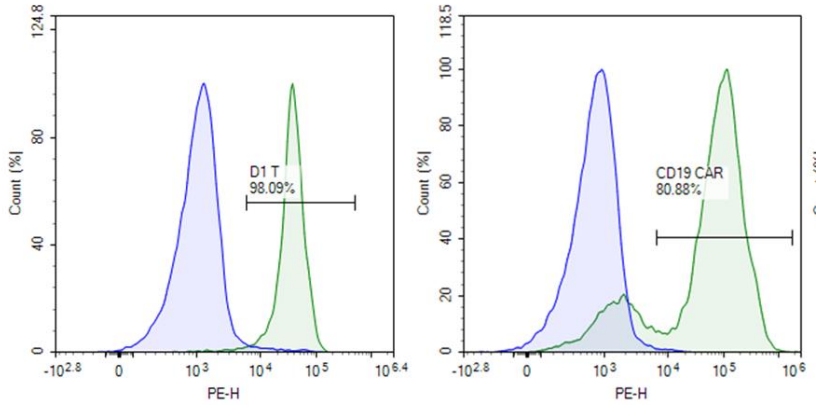

Figure 2: Expression of anti-CD19 CAR in CAR-Gamma Delta T Cells (δ1) expanded with Delta 1 (Vδ1) T Cell Expansion System.
20-day expanded CAR-Gamma Delta T cells (δ1) (green) and control cells (blue) were thawed 24 hours prior to the experiment. Approximately 50,000 cells were analyzed by flow cytometry using Fluosite™Anti-TCRγδ1 (gamma delta 1) Antibody, PE-Labeled (#102760) (Left panel, Blue: Untransduced Gamma Delta T cells(δ2) (#82498) were used as negative control) and PE-anti-FMC63 ScFv (Acrobiosystems #FM3-HPY53-25tests) (Right panel, Blue: Untransduced Gamma Delta T cells(δ1) (#82496) were used as negative control). The y axis represents the % of cells, while the x axis indicates the PE intensity.

Functional Validation

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 (δ1) gently (300 *x g* for 5 minutes) and resuspend in Gamma Delta T Cells Medium.

3. Prepare Anti-CD19 CAR-Gamma Delta T cells (δ1) 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 (δ1) 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 (Room Temperature) 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 Untransduced Gamma Delta T Cells (δ1) (#82496) 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 (δ1) gently (300 *x g* for 5 minutes) and resuspend in Gamma Delta T Cells Medium.

3. Prepare Anti-CD19 CAR-Gamma Delta T cells (δ1) 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 (δ1) into the "Test" wells, which contain target cells, at the desired effector:target (E:T) cell ratio.

5. Add 50 µl of Anti-CD19 CAR-Gamma Delta T Cells Medium to the "No T Cell Control" wells.

6. Add 100 µl of Anti-CD19 CAR-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 T Cell Control" wells).

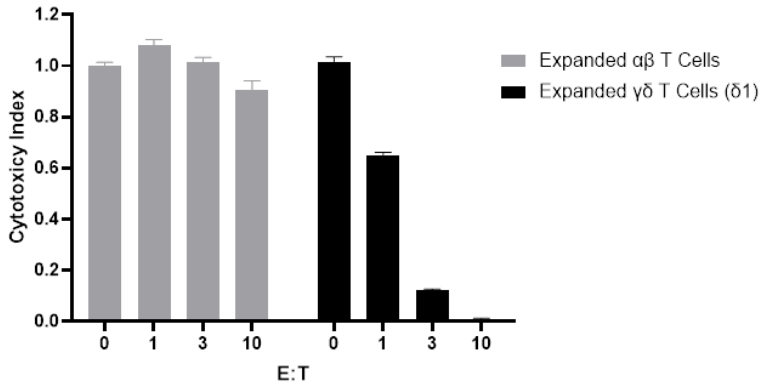

Figure 3: Expanded human peripheral blood Gamma Delta T cells cytotoxicity assay using Firefly Luciferase K562 Cell Line as target cells.
14-day expanded human peripheral blood Alpha Beta (αβ) and Gamma Delta (γδ) T cells were thawed for 24 hours prior to the experiment. T cells (effector) were then co-cultured with Firefly Luciferase K562 cells for 24 hours at the indicated effector:target (E:T) ratio. The lysis of target cells was determined by measuring luciferase activity with ONE-Step™ Luciferase Assay System (#60690).

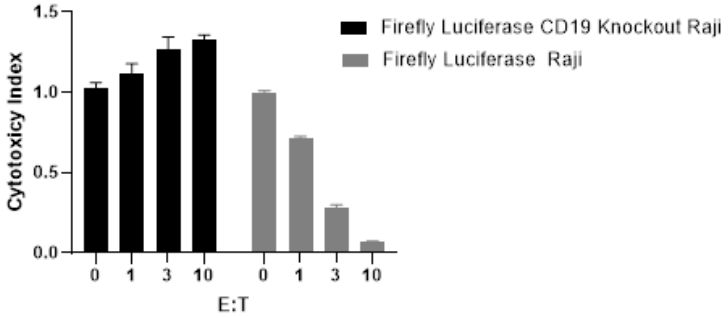

Figure 4: Luciferase-based CD19-specific cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells.
Anti-CD19 CAR- Gamma Delta T Cells (δ1) were thawed 48 hours prior to the experiment. These cells (effector) were then co-cultured with Firefly Luciferase Raji cells and Firefly Luciferase CD19 Knockout Raji cells for 24 hours at the indicated effector:target (E:T) ratios. 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*
Untransduced Gamma Delta T Cells (δ1)	82496	1 vial
Anti-CD19 CAR-Gamma Delta T Cells (δ1)	82497	1 vial
Expanded Human Peripheral Blood Gamma Delta T Cells (δ1), Frozen	82443	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 052725