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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2), Frozen are Vγ9Vδ2 T cells enriched and expanded from human PBMCs (Peripheral Blood Mononuclear Cells) using the Vγ9Vδ2 T Cell Expansion Kit (BPS Bioscience #82551), and cryopreserved. Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2), Frozen are >90 % pure Vγ9Vδ2 T cells ($CD3^+$ and TCR $V\gamma9^+$cells), as measured by flow cytometry analysis. They can be used in cytotoxicity assays and other *in vitro* assays, after thaw in T Cell Medium (#78753).

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, with Vγ9Vδ2 being the predominant γδ T cell type in human peripheral blood. Vγ9Vδ2 T cells are involved mostly in immune responses to pathogens and long-term modulation of inflammation, and can recognize non-peptide phospho-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(s)

- Use in several *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

Component	Format
1 vial of frozen cells	Vial contains 2 x 10^6 cells in CryoStor® CS10 (Stemcell Technologies #100-1061)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

Storage Conditions

Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) 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 cells but are necessary for setting up a γδ T cell cytotoxicity assay. BPS Bioscience's reagents are validated and optimized and are highly recommended for the best results.

Materials required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2D	BPS Bioscience #79639
TCellM™	BPS Bioscience #78753
Human Interleukin-2 Recombinant	BPS Bioscience #90184
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	
96 Well White, Clear Bottom Plate	
Activated human αβ T Cells	

Media Formulations

For best results, the use of these validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for Functional Cellular Assay

Assay Medium:

TCellM™ (#78753) supplemented with 1000 IU/ml of Human Interleukin-2 Recombinant (#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.

Cell Thawing Protocol

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.
2. 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 content of the vial to a tube containing 10 ml of pre-warmed TCellM™.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in appropriate assay medium. The cells are ready to be used in the desired applications.

Validation Data

- This protocol is a general guideline only for evaluating the cytotoxicity of the frozen Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) in an *in vitro* assay.
- This protocol is designed to perform cytotoxicity assays in a 96-well plate. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

- Use TCellM™ (#78753) supplemented with 1000 IU/ml of Human Interleukin-2 Recombinant (#90184) for Assay Medium.
- Firefly Luciferase K562 Cell Line (#78621) and Firefly Luciferase Raji Cell Line (#78622) are used as target cells. Maintenance conditions can be found in the respective datasheets at bpsbioscience.com.
- Cryopreserved γδ T cells can be thawed and immediately used in any *in vitro* assays. Expanding frozen γδ T cells is generally not recommended, as it can lead to a substantial reduction in their proliferation rate and/or cytotoxic activity, depending on the PBMC donor used for the expansion.
- Unlike conventional αβ T cells, γδ T cells can kill target cells without requiring antibodies although they can be used in combination with γδ T cell engagers.
- Conditions should be run in triplicate.
- If performing cytotoxicity assays without antibodies the assay should include the following experimental conditions:
    - αβ T Cell Control: This control consists of both activated αβ T cells and target cells and serves as a negative control. Activated human αβ T cells are not provided and must be prepared by the client using their preferred method.
    - No Target Cell, αβ T Cell Control: This control contains only αβ T cells cell and used to determine the background luminescence signal.
    - No Target Cell, γδ T Cell Control: This control contains only γδ T cell and used to determine the background luminescence signal.
    - γδ T Cell Test: Contains both γδ T Cell and target cells used to measure cytotoxicity of the γδ T cell towards the target cells in the absence of the γδ T Cell engagers.

One week prior to running the assay: Target Cells Thaw and Expansion

Cell Thawing

1. Retrieve a vial of Firefly Luciferase K562 Cell Line and/or Firefly Luciferase Raji Cell Line from liquid nitrogen storage. Keep on dry ice until ready to thaw.
2. Swirl the vials 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 content 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.
3. 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 Thaw Medium 2.
4. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
6. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2, and continue growing in a 5% CO2 incubator at 37°C until the cells are ready to passage.

   Note: Cells should be passaged before they reach a density of 2 x 10^6. At first passage and subsequent passages, use Growth Medium 2D.

Cell Passage

Dilute the cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml in Growth Medium 2D. The sub-cultivation ratio should be approximately 1:5 to 1:10 once or twice a week, so cells are maintained between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Day 1: Assay Setup

For 96-well plate assays, each well will contain a final volume of 100 µl (50 µl of expanded γδ T cells at the desired E:T ratio and 50 µl of target cells).

1. Transfer 2 x 10^6 target cells to a clean 15 ml tube and centrifuge at 300 *x g* for 5 minutes.
2. Aspirate supernatant and resuspend cells in 10 ml of Assay Medium (2 x 10^5 cells/ml).
3. Transfer cells to a solution reservoir.
4. Using a multichannel pipette, transfer 50 µl of the target cell suspension (10,000 cells/well) to the "αβ T Cell Control" and "γδ T Cell Test" wells.
5. Using a multichannel pipette, transfer 50 µl of Assay Medium to the "No Target Cell, γδ T Cell Control" and "No Target Cell, αβ T Cell Control" wells.
6. Keep the plate in a humidified 37°C incubator with 5% CO_2 while preparing γδ T cells.

Thaw Human Peripheral Blood Gamma Delta T Cells

4. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.
5. 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 content of the vial to a tube containing 10 ml of pre-warmed TCellM™.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

6. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in pre-warmed Assay Medium to 4 x 10^6 cells/ml for an E:T ratio 20:1.
7. Prepare the activated human αβ T cells using the selected method and resuspend them in pre-warmed Assay Medium at a concentration of 4 x 10^6 cells/ml for an E:T ratio of 20:1.

   Note: E:T ratio may need to be optimized in different experimental settings and cell density may need to be adjusted.

8. Add 50 µl of γδ T cell suspension to "γδ T Cell Test", and "No Target Cell, γδ T Cell Control" wells.
9. Add 50 µl of αβ T Cells suspension to "αβ T Cell Control" and "No Target Cell, αβ T Cell Control" wells.
10. Incubate the assay plate for 24 hours in a humidified 37°C incubator with 5% CO_2.

Note: The incubation time may need to be optimized for your assay.

Day 2: Luciferase Analysis

1. Thaw Luciferase Reagent Buffer (Component A) by placing the reagent in a Room Temperature (RT) water bath.

2. Equilibrate the buffer to RT and mix well before use.
3. Immediately before the experiment, prepare the Luciferase Assay Working Solution by diluting Luciferase Reagent Substrate (Component B) 100-fold with Luciferase Reagent Buffer (Component A), and mix well (you will need 100 µl/well).

   Note: Avoid exposure to excessive light. Only use enough of each component for the experiment, and store the remaining Component A and Component B separately at -20°C.
4. Remove the cells from the incubator and add 100 µl of Luciferase Assay Working Solution directly to the culture medium of each well.
5. Wrap the plate with foil and gently rock it for ≥15 minutes at RT.
6. Measure firefly luminescence using a luminometer.

Validation Data

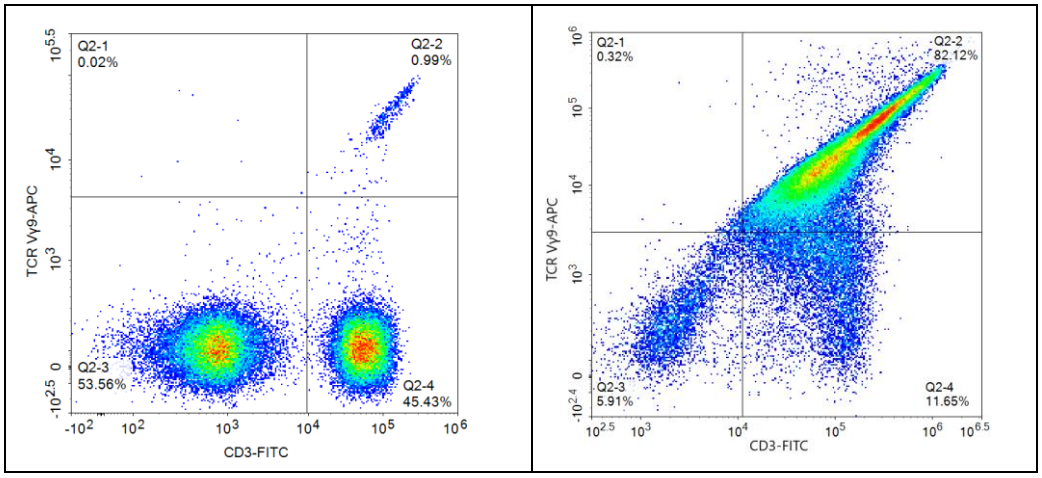

Figure 1: γδ T cell marker assessment of the Frozen Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) by flow cytometry.
Frozen Normal Human Peripheral Blood Mononuclear Cell, PBMCs, (BPS Bioscience # 79059) and Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) (BPS Bioscience # 82733) were thawed and immediately stained with APC-labeled anti-human TCR Vγ9 Antibody (BioLegend #331310) and FITC-labeled anti-human CD3 Antibody (BioLegend #300406) and analyzed by flow cytometry. Representative flow cytometry plots show the percentage of Vγ9Vδ2 T cells ($CD3^+$ and TCR $V\gamma9^+$) and αβ T cells ($CD3^+$ and TCR $V\gamma9^-$) in the PBMCs (left) and Expanded Human Peripheral Blood Gamma Delta T Cells (right). Each plot was gated on FSC-A/SSC-A (to remove debris from analysis) and FSC-H/FSC-A (singlet discrimination) (not shown).

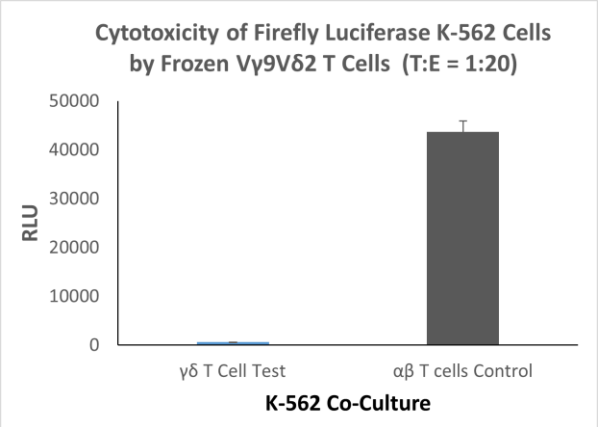

Figure 2: Luciferase-based cytotoxicity measure of Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) co-cultured with Firefly Luciferase K-562 Cell Line.
Frozen Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) was thawed and co-cultured with Firefly Luciferase K-562 cells (#78621) for 24 hours at a 20:1 ratio in a 96-well white, clear bottom plate. As control, Firefly Luciferase K-562 cells were co-cultured with activated human αβ T cells. After incubation, luciferase activity was detected with One-Step™ Luciferase Assay System (#60690). A reduction in the raw bioluminescence signal results from the cytotoxicity activity of Vγ9Vδ2 T cells.

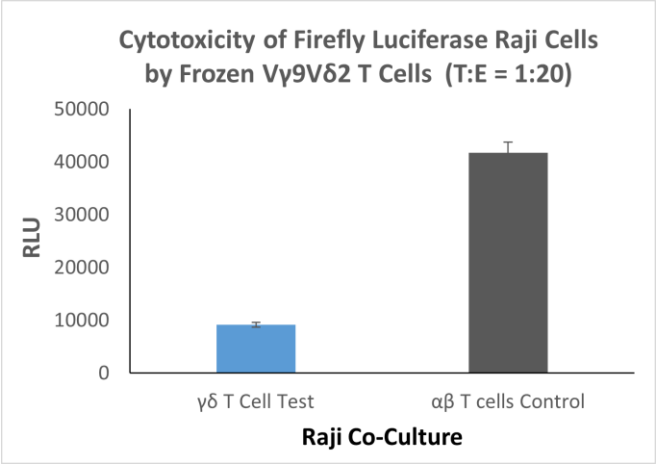

Figure 3: Luciferase-based cytotoxicity measure of Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) co-cultured with Firefly Luciferase Raji Cell Line.
Frozen Expanded Human Peripheral Blood Gamma Delta T Cells (Vγ9Vδ2) was thawed and co-cultured with Firefly Luciferase Raji cells (#78622) for 24 hours at a 20:1 ratio in a 96-well white, clear bottom plate. As control, Firefly Luciferase Raji cells were co-cultured with activated human αβ T cells. After incubation, luciferase activity was detected with One-Step™ Luciferase Assay System (#60690). A reduction in the raw bioluminescence signal results from the cytotoxicity activity of Vγ9Vδ2 T cells.

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

Troubleshooting Guide
For all further questions, please email support@bpsbioscience.com

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

Related Products

Products	*Catalog #*	*Size*
Vγ9Vδ2 T Cell Expansion Kit	82551	1 kit
Vγ9Vδ2 TCR Lentivirus	78985	100 µl/500 µl x 2
Vγ9Vδ2 TCR NFAT-Luciferase Reporter Jurkat Cell Line	82320	2 vials
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78556	2 vials
Vγ4Vδ1 TCR Lentivirus	78986	100 µl/500 µl x 2
Vγ4V12 TCR NFAT-Luciferase Reporter Jurkat Cell Line	82329	2 vials

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