Description

Vγ9Vδ2 T Cell Expansion Kit is suitable for the *ex vivo* culture and expansion of human Vγ9Vδ2 T cells. γδ T cells are low abundance in PBMCs (< 2%) and therefore they need to be successfully activated and expanded to obtain adequate number of cells for γδ T-cell based immunotherapy related studies. This kit contains media and reagents necessary to drive the robust activation and expansion of the Vγ9Vδ2 T cells subpopulation from PBMCs. To improve the purity of the expanded cells, αβ T cells and B cells are depleted from the expansion culture using an antibody cocktail. This kit is provided with enough reagents and materials for the activation and expansion of Vγ9Vδ2 T cells from a starter population of 10 x 10^7 PBMCs.

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)

Activation and expansion of Vγ9Vδ2 T cell from freshly isolated or frozen PBMCs, for downstream applications in CAR (chimeric antigen receptor) γδ T-cell development, such as cytotoxicity assays and flow cytometry.

Supplied Materials

Catalog #	Name	Amount	Storage
90184	Human Interleukin-2 Recombinant	50 µl	-80°C
78753	TCellM™	100 ml	-20°C
	Activation Reagent (5000X)	10 µl	-80°C
	Cell Isolation Magnetic Beads	150 µl	2-8°C
	Cell Depletion Antibody Cocktail	100 µl	2-8°C
78563	5x Cell Isolation Buffer	25 ml	-20°C

Storage Conditions

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

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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.

Name	Ordering Information
Normal Human Peripheral Blood Mononuclear Cells, Frozen	BPS Bioscience #79059
Thaw Medium 2	BPS Bioscience #60184
Firefly Luciferase NALM6 Cell Line	BPS Bioscience #78494
CryoStor® CS10	STEMCELL TECHNOLOGIES #07930
Falcon® 5 ml Round Bottom Polystyrene Test Tube	Corning #352054
EasyEights™ EasyStep™ Magnet	STEMCELL TECHNOLOGIES #18103
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
APC anti-human TCR Vγ9 Antibody	BioLegend #331310
FITC anti-human CD3 Antibody	BioLegend #300406
Luminometer	

Media Formulations

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

γδ T Cell Activation Medium:

TCellM™ (#78753) + 500 IU/ml of Human Interleukin-2 Recombinant (#90184) + 1x Activation Reagent.

γδ T Cell Expansion Medium:

TCellM™ (#78753) + 500 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.

Vγ9Vδ2 T-Cell Expansion Protocol:

- The following protocol was designed as a general guideline for the expansion of Vγ9Vδ2 T cells from 5 x 10^6 PBMCs. To perform expansion of different cells numbers, the reagent and media volume should be scaled appropriately.
- This protocol has been optimized for use with Normal Human Peripheral Blood Mononuclear Cells, Frozen (#79059). If using PBMCs from another vendor, it may require optimization.
- The expansion fold obtained will vary, depending on the source of cells and donor.
- Dilute 5x Cell Isolation Buffer 5-fold with sterile water to make 1x Cell Isolation Buffer. Further sterile filtration is optional. Approximately 20 ml of diluted 1x Cell Isolation Buffer is required for every 5 x 10^6 PBMCs.
- To maintain optimal conditions, it is recommended to practice aseptic techniques and work as quickly as possible. Mixing cells thoroughly during the cell depletion step is critical to obtain high cell purity.
- All steps should be performed at room temperature unless otherwise specified.

Vγ9Vδ2 T-Cell Activation

1. If using frozen PBMC, thaw 5 x 10^6 cells for 1-2 minutes in a 37°C bath and transfer the cells to a 15 ml tube containing 10 ml of warm Thaw Medium 2.
2. Spin down at 300 *x g* for 5 minutes.
3. Discard the supernatant and resuspend cells in 5 ml of γδ T Cell Activation Medium (1 x 10^6 cells/ml).
4. Transfer cells to a well in a 6-well plate and incubate in a humidified 37°C incubator with 5% CO_2 for 72 hours.
5. On day 3, transfer cells to a 15 ml tube.
6. Rinse the well with Thaw Medium 2 to collect any remaining cells and transfer to the same tube.
7. Spin down the tube for 5 minutes at 300 *x g*.
8. Aspirate the supernatant and resuspend the cell pellet in 10 ml of γδ T cell Activation Medium.
9. Transfer cells to a T-25 flask and incubate in a humidified 37°C incubator with 5% CO_2 for 72 hours.

αβ T-Cell and B-Cell Depletion

1. On day 6, transfer cells from the T-25 flask to a 15 ml tube.
2. Rinse the T-25 flask with Thaw Medium 2 to collect any remaining cells and transfer to the same tube.
3. Spin down cells at 300 *x g* for 5 minutes.
4. Aspirate the supernatant and resuspend the cell pellet in 4 ml of 1x Cell Isolation Buffer.
5. Transfer cells to a Falcon® 5 ml tube.
6. Spin down the tube at 300 *x g* for 5 minutes.
7. Aspirate the medium and resuspend the cell pellet in 250 µl of 1x Cell Isolation Buffer by gently pipetting 5-7 times or until cell clumps are broken completely.
8. Add 50 µl of the Cell Depletion Antibody Cocktail directly to the cells. Gently pipet to mix well.
9. Incubate the cell-antibody suspension on a shaker at Room Temperature (RT) for 15 minutes. Flick the tubes periodically to ensure that the cells are properly mixed throughout the incubation.
10. Add 2 ml of 1x Cell Isolation Buffer and pipet to mix well.
11. Spin down cells at 300 *x g* for 5 minutes.

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12. Discard the supernatant and resuspend the cells in 150 µl of 1x Cell Isolation Buffer by pipetting 5-7 times or until cell clumps are broken completely.

13. Wash 75 µl of Cell Isolation Magnetic Beads with 1 ml of 1x Cell Isolation Buffer.

14. Place the tube with the beads on the magnet for 3 minutes.

15. Carefully remove the buffer without disturbing the beads.

16. Resuspend the beads in 150 µl of 1x Cell Isolation Buffer.

17. Add 150 µl of washed beads to the 150 µl of cell suspension. Gently pipet 5-7 times to mix well.

18. Incubate for 10 minutes on a shaker at RT. Flick the tubes periodically to ensure that the beads/cells are properly mixed throughout the incubation.

19. Add 1.2 ml of 1x Cell Isolation Buffer and gently mix by pipetting.

20. Place the tube on the magnet for 5 minutes.

21. Transfer the supernatant (containing γδ T cells), gently in order to avoid transferring the beads, into a new 5 ml tube. Count cells and calculate the total number of recovered cells.

Vγ9Vδ2 T Cell Expansion

1. Spin down the tube for 5 minutes at 300 *x g*.

2. Aspirate the supernatant and resuspend the cell pellet in complete γδ T Cell Expansion Medium at 0.6 x 10^6 cells/ml.

3. Transfer cells to a T-25 flask and incubate in a humidified 37°C incubator with 5% CO_2 for 72 hours.

4. On day 9, transfer cells to a 15 ml tube. Rinse the flask with Thaw Medium 2 to collect the remaining cells and transfer to the same tube.

5. Spin down the tube for 5 minutes at 300 *x g*.

6. Aspirate the supernatant and resuspend the cell pellet in 25 ml of γδ T Cell Expansion Medium.

7. Transfer cells to a T-75 flask and incubate in a humidified 37°C incubator with 5% CO_2 for 72 hours.

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8. On day 12, cells are ready to be used for any downstream assay such as γδ T cell cytotoxicity assays. Alternatively, they can be cryopreserved for future applications.

   Note: CryoStor® CS10 cryopreservation medium from STEMCELL TECHNOLOGIES is recommended for maximizing post-thaw cell recovery and viability of γδ T cells. Please check the manufacturing instructions for a detailed cryopreservation protocol.

   Thawed γδ T cells need to be recovered for 24 hours with fresh complete γδ T Cell Expansion Medium in a humidified 37°C incubator with 5% CO_2 before being used in any cytotoxicity assays.

Validation Data

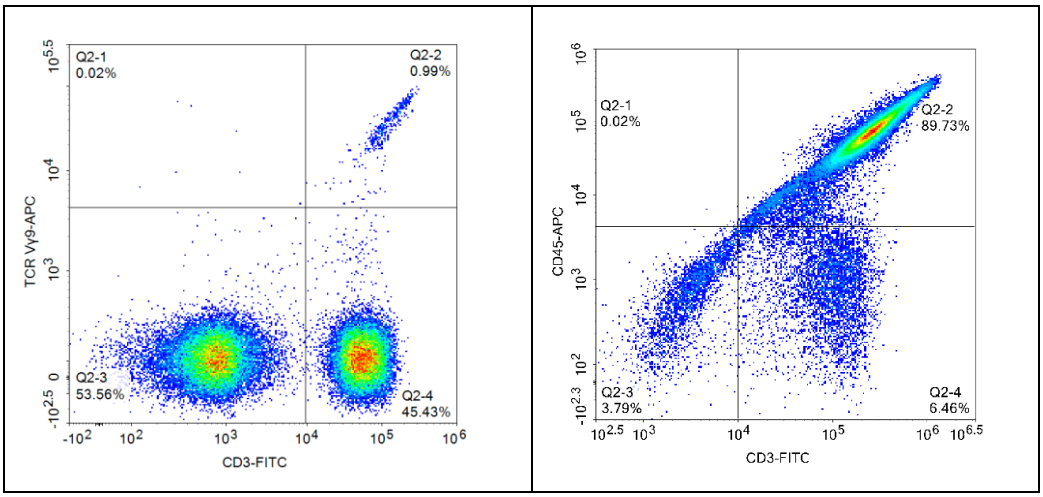

Figure 1: γδ T cell marker assessment on expanded Vγ9Vδ2 T cells by flow cytometry.
PBMCs (BPS Bioscience #79059) were stimulated with γδ T Cell Activation Reagent and expanded for 12 days in complete T Cell Expansion Medium. αβ T cells and B cells were depleted on day 6. The purity of the expanded Vγ9Vδ2 T cell population was determined by flow cytometry. Cells were stained with APC anti-human TCR Vγ9 Antibody (BioLegend #331310) and FITC 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 $Vγ9^+$) and αβ T cells ($CD3^+$ and TCR $Vγ9^-$) in expanded cells at day 0 (left) and day 12 (right).

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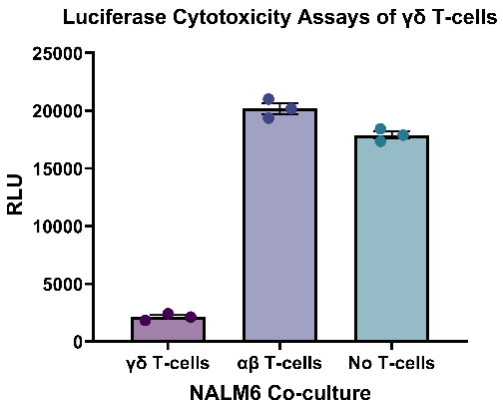

Figure 2: Luciferase-based cytotoxicity measure of expanded Vγ9Vδ2 T cells co-cultured with Firefly Luciferase NALM6 Cell Line.
Vγ9Vδ2 T cells obtained after a 12-day expansion period and Firefly Luciferase NALM6 cells (#78494) were co-cultured for 24 hours at a 10:1 ratio in a 96-well white, clear bottom plate. As controls, Firefly Luciferase NALM6 cells were cultured alone or co-cultured with activated 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 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
PBMC Cytotoxicity Luciferase Assay Kit (NALM6)	82174	1 Kit

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