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Instructions for Culturing Gamma Delta (γδ) T Cells Using TheraPEAK® T-VIVO® Cell Culture Medium

I. Introduction

TheraPEAK® T-VIVO® Cell Culture Medium

TheraPEAK® T-VIVO® Cell Culture Medium is optimized to support cell therapy applications utilizing human T cells. It only uses recombinant human proteins and does not require serum or serum substitute. TheraPEAK® T-VIVO® Cell Culture Medium is manufactured in compliance with cGMP and all ingredients are chemically-defined and non-animal origin (NAO). It does not contain cytokines, antibiotics or phenol red.

Gamma delta (γδ) T cells

Gamma delta (γδ) T cells represent a relatively small subset of T cells and are defined by the expression of heterodimeric T-cell receptors (TCRs) composed of γ and δ chains, and can be further identified by different Vδ (variable δ) chains. For example, Vδ1 T cells are predominant in the peripheral tissues and Vδ2 T cells constitute the majority of blood γδ T cells.

γδ T cells have attractive properties for cancer immunotherapy including their inherent ability to infiltrate tissues and to directly recognize and kill transformed cells independent of HLA-antigen presentation. Moreover, γδ T cells provide a platform for allogeneic cell therapies as they do not cause graft-versus-host disease (GVHD).

The first protocol describes a process that uses zoledronic acid as the activating agent and peripheral blood mononuclear cells (PBMCs) as the starting cell source for Vδ2 T cells expansion, using TheraPEAK® T-VIVO® Medium without the addition of human serum. Vδ2 T cells can be expanded by more than 1,000-fold in 14 days using this protocol.

A second protocol describes an isolation process that is tailored to deplete αβ T cells from PBMCs and then uses anti-CD3/anti-CD28 co-stimulation to activate and expand γδ T cells (Vδ1 and Vδ2 T-cell subsets) using the TheraPEAK® T-VIVO® Medium.

II. Storage

TheraPEAK® T-VIVO® Cell Culture Medium should be stored at 2 to 8 °C, protected from light.

III. Instructions for use

Media preparation

TheraPEAK® T-VIVO® Cell Culture Medium may be supplemented with cytokines such as with the TheraPEAK® AmpliCell® recombinant IL-2. The amount of cytokines required may vary depending on the user applications, however for this protocol, it is suggested to use 100 IU/mL of recombinant human IL-2. The medium with cytokine may be stored at 2 to 8 °C for up to 7 days. When in use, minimize exposure of medium to light.

General guideline for Vδ2 T-cells activated with zoledronic acid and cultured in T-flasks

For optimal gas exchange in static T-flasks, it is recommended that the medium height be less than 3mm.

1. Prepare fresh peripheral blood mononuclear cells (PBMCs) or thaw frozen PBMCs in a 37 °C water bath according to standard thawing protocols.

2. Wash the cells with TheraPEAK® T-VIVO® Cell Culture Medium.
3. Centrifuge the cells at 200 – 300 x g for 5–10 minutes and remove wash buffer.
4. Resuspend the cells in TheraPEAK® T-VIVO® Cell Culture Medium with cytokine. Determine viable cell concentration and cell viability using standard cell counting protocols.
5. Plate 2.0 x 10e6 viable PBMCs in 1 mL TheraPEAK® T-VIVO® Cell Culture Medium with cytokine into one well of a 24-well plate for small scale expansion. For larger cell numbers, plate at 2.0 x 10e6 viable PBMCs/mL of medium in the appropriate vessel to allow for sufficient gas exchange.
6. Stimulate Vδ2 T cells by adding zoledronic acid to final concentration of 5 – 10 µM.
7. Incubate the culture vessel at 37 °C in a humidified incubator with 5% CO_2.

   Every 2 – 3 days, change 50% of the medium.
8. When the cells become confluent, transfer the entire culture into a larger vessel and add fresh medium to the appropriate volume.
9. Continue to expand the T-cell culture by adding fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine every 2 – 3 days and re-adjusting the cell density to 1.0 x 10e6 viable cells/mL. Use larger T-flasks as needed.
10. Harvest cells when the desired cell number is achieved and proceed to downstream applications.

General guideline for γδ T-cell expansion with anti-CD3/anti-CD28 co-stimulation after depleting αβ T cells from PBMCs

Both γδ T cells and αβ T cells express CD3 and CD28, hence it is critical to deplete αβ T cells prior to expansion using this protocol. αβ T cells may be removed from PBMCs with a variety of commercial cell separation products.

1. Prepare fresh peripheral blood mononuclear cells (PBMCs) or thaw frozen PBMCs in a 37 °C water bath according to standard thawing protocols.
2. Wash the cells with TheraPEAK® T-VIVO® Cell Culture Medium.
3. Centrifuge the cells at 200 – 300 x g for 5 – 10 minutes and remove wash buffer.
4. Resuspend the cells in TheraPEAK® T-VIVO® Cell Culture Medium. Determine viable cell concentration and cell viability using standard cell counting protocols.
5. If start with cryopreserved PBMCs, allow PBMCs recover in TheraPEAK® T-VIVO® Cell Culture Medium overnight at 37 °C in a humidified incubator with 5% CO_2, before isolating the γδ T cells.
6. Isolate γδ T cells from PBMCs.

   A variety of commercial cell separation products may be used to isolate γδ T cells from PBMCs. Non-γδ T cells, i.e. αβ T cells, NK cells, B cells, dendritic cells, granulocytes, monocytes, stem cells etc. may be depleted using a cocktail of antibodies. At minimum, αβ T cells should be depleted using αβTCR-specific antibodies.
7. Determine viable cell concentration of the isolated γδ T cells using standard cell counting protocols.
8. For research purposes, plate 0.5 – 1.0 x 10e6 viable γδ T cells in 1 mL TheraPEAK® T-VIVO® Cell Culture Medium with cytokine into one well of a 24-well plate. Alternatively, a 96-well plate may be used, in which case, plate 5.0 – 10.0 x 10e3 viable cells in one well of a 96-well plate in 150 µL medium.

   If only αβ T cells are depleted from PBMCs, plate 1.0 – 2.0 x 10e6 viable cells in 1 mL TheraPEAK® T-VIVO® Cell Culture Medium with cytokine into one well of a 24-well plate for small scale expansion.
9. Stimulate T cells for expansion using commercial anti-CD3 and anti-CD28 T cell activation products as recommended by the suppliers. For example, use 10µL T Cell TransAct™ (Miltenyi) for 1 mL medium.
10. Incubate the culture vessel at 37 °C in a humidified incubator with 5% CO_2.
11. Continue to expand the T-cell culture by adding fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine every 2 – 3 days.

Count the cells if possible and re-adjust the cell density to 0.5 – 1.0 x 10e6 viable cells/mL. Use larger T-flasks as needed.

12. Harvest cells when the desired cell number is achieved and proceed to downstream applications.

IV. General recommendations

Refer to the general TheraPEAK® T-VIVO® Cell Culture Medium instruction for recommendations on adopting processes that maintain good dissolved oxygen levels during the cell expansion processes, and optimizing processes that involve Nucleofection® or electroporation.

Lentivirus transduction in γδ T cells

Lentivirus pseudotyped with the common VSV-G envelope protein enters cells using the low-density lipoprotein receptor (LDL-R) as the major entry receptor, however, LDL-R expression is very low in resting T cells. Stimulation of T cells through the T-cell receptor upregulates the LDL-R expression within 24-72 hours after stimulation, and permits efficient VSV-G pseudotyped lentivirus transduction. Therefore, for optimal lentivirus transduction of γδ T cells, it is recommended to activate the cells by crosslinking CD3 and CD28 followed by lentivirus transduction between 24 and 72 hours after activation.

Product use statement

All T-VIVO® products are produced according to applicable GMP standards and follow the USP/EP guidance for cell and gene therapy raw materials. Lonza Group Ltd. and its affiliates (collectively and individually, "Lonza") make efforts to include accurate and up-to-date information. However, Lonza makes no representations or warranties, express or implied, including as to accuracy or completeness of information. All trademarks belong to Lonza, and are registered in the USA, EU and/or CH, or used in common law, or belong to third-party owners and are used for only informational purposes. All third-party copyrights have been reproduced with permission from their owners. The user bears the sole responsibility for determining the existence of any third-party rights and obtaining any necessary licenses and approvals. For more information, including regarding legal disclaimers, Lonza's intellectual property rights, and how Lonza collects, uses and protects personal information: www.lonza.com/legal, https://www.lonza.com/about-us/strategy/intellectual-property, and www.lonza.com/privacy.