

## TheraPEAK® T-VIVO® Cell Culture Medium

### Instructions for use

---

#### I. Introduction

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.

#### II. Storage

TheraPEAK® T-VIVO® Cell Culture Medium should be stored at 2–8 °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, IL-7 or IL-15. The amount of cytokines required may vary depending on the user applications. For standard T cell expansion, 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 10 days. When in use, minimize exposure of medium to light.

TheraPEAK® T-VIVO® Cell Culture Medium is designed to support T cell culture without the addition of serum or serum substitute. If user decides to add serum or serum substitute, the

amount required should be determined empirically depending on the specific T cell application.

##### General guideline for T cell culture in T-flask

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

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

T cells may be isolated from PBMCs for subsequent expansion. A variety of commercial cell separation products may be used to isolate T cells from PBMCs.

2. Wash the cells with TheraPEAK® T-VIVO® Cell Culture Medium with cytokine.
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 the required number of cells into appropriate tissue culture vessel.

For example, plate 1.0 x 10<sup>6</sup> viable PBMCs or 0.5 x 10<sup>6</sup> viable T cells in 1 mL TheraPEAK® T-VIVO® Cell Culture Medium with cytokine into one 24-well.

6. Stimulate T cells for expansion using a variety of commercial anti-CD3 and anti-CD28 T cell activation products as recommended by the suppliers.
7. Incubate the culture vessel at 37 °C in a humidified incubator with 5% CO<sub>2</sub>.  
On day 1 or 2, lentivirus or retrovirus carrying gene-of-interest may be added for transduction.

- On day 2 or 3, transfer the cells from 24-well into a T-25 flask for expansion.  
Remove the T cell activation product if desired, according to protocols recommended by the suppliers.
- Continue to expand the T cell culture by adding fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine every 2–3 days and re-adjust the cell density to 0.5–1.0 x 10<sup>6</sup> viable cells/mL. Use larger T-flasks as needed for cell expansion.
- Over 10–14 days in static culture with T-flasks, several hundreds to over one thousand fold expansion of human T cells may be achieved.
- Harvest cells when the desired cell number is achieved and proceed to downstream application, e.g., analysis of cells.
- Stimulate T cells for expansion using a variety of commercial anti-CD3 and anti-CD28 T cell activation products as recommended by the suppliers.
- Incubate G-Rex®6 Well Plate at 37 °C in a humidified incubator with 5% CO<sub>2</sub>.
- On day 2 or 3, add fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine to the maximum capacity of G-Rex®6 Well Plate (40 mL).  
Remove the T cell activation product if desired, according to protocols recommended by the suppliers.
- G-Rex® holds a sufficient amount of medium and may not require medium exchange for 4–5 days, provided that cytokine (e.g., IL-2) is added every 2–3 days as recommended.

If desired, on day 4 or 5, aspirate and discard 75% of the medium from G-Rex®6 Well Plate. Cells remain on the gas permeable membrane so long as the aspirating pipette remains near the top of the medium during this process. In G-Rex®6 Well Plate, 75% of the medium is 30 mL.

Swirl the remaining medium to dislodge the cells from the gas permeable membrane and re-suspend them in the medium. Determine total viable cell number and cell viability using standard cell counting protocols.

Add fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine to the maximum capacity in G-Rex®6 Well Plate (40 mL) and continue cell expansion.

### General guideline for T cell culture in G-Rex®

In G-Rex®, the cells reside on a gas permeable silicone membrane for optimal gas exchange. G-Rex® holds a sufficient amount of medium and may not require medium exchange for 4–5 days, provided that cytokine (e.g., IL-2) is added every 2–3 days as recommended.

- Prepare fresh peripheral blood mononuclear cells (PBMCs) or thaw frozen vials of PBMCs in a 37 °C water bath according to standard thawing protocols.  
T cells may be isolated from PBMCs for subsequent expansion. A variety of commercial cell separation products may be used to isolate T cells from PBMCs.
- Wash the cells with TheraPEAK® T-VIVO® Cell Culture Medium with cytokine.
- Centrifuge the cells at 200–300 x g for 5–10 minutes and remove wash buffer.
- Resuspend the cells in TheraPEAK® T-VIVO® Cell Culture Medium with cytokine. Determine viable cell concentration and cell viability using standard cell counting protocols.
- Plate 0.5–1.0 x 10<sup>6</sup> viable cells/cm<sup>2</sup> into G-Rex®6 Well Plate.  
For example, plate 5.0–10.0 x 10<sup>6</sup> viable cells in G-Rex®6 Well Plate (10 cm<sup>2</sup> surface) in 3–5 mL the medium with cytokine.
- Check cell growth every 2–3 days until the cells reach the typical maximum capacity, which is about 300 million cells in G-Rex®6 Well Plate.  
If desired, the cells can be transferred into G-Rex® with larger surface area to continue expansion.
- Harvest cells when the desired cell number is achieved and proceed to downstream application, e.g., analysis of cells.

## General guideline for T cell culture in spinner flask

The spinner flask comes ready-to-use with a paddle and integrated magnet, which provides smooth, even rotation at required speeds for suspension cell culture on slow-speed magnetic stirring platform.

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

T cells may be isolated from PBMCs for subsequent expansion. A variety of commercial cell separation products may be used to isolate T cells from PBMCs.

2. Wash the cells with TheraPEAK® T-VIVO® Cell Culture Medium with cytokine.
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. Seed the required number of cells into an appropriate size spinner flask.  
For example, seed 1.0–2.0 x 10<sup>6</sup> viable cells per mL in TheraPEAK® T-VIVO® Cell Culture Medium with cytokine into a 125 mL spinner flask, with 15–20 mL medium.
6. Stimulate T cells for expansion using a variety of commercial anti-CD3 and anti-CD28 T cell activation products as recommended by the suppliers.
7. Loosen the caps on the spinner flask side arms to allow gas exchange. Incubate the spinner flask at 37 °C in a humidified incubator with 5% CO<sub>2</sub>.  
In the first 2–3 days, it is recommended to culture the cells in the spinner flask without agitation during the activation phase.
8. On day 2 or 3, remove the T cell activation product if desired, according to protocols recommended by the suppliers.

9. Determine total viable cell number and cell viability using standard cell counting protocols on a daily basis. Add fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine to re-adjust the cell density to 1.0 x 10<sup>6</sup> viable cells/mL in the spinner flask.
10. Put the spinner flask on a slow-speed magnetic stirring platform and set appropriate speed to start culturing the cells in suspension, inside a humidified incubator with 5% CO<sub>2</sub> at 37 °C.
11. Repeat step 9 daily until reaching the maximum volume in the spinner flask (100 mL in a 125 mL spinner flask).
12. Start daily medium exchange. Centrifuge the cells at 300 x g for 5–10 minutes to remove spent medium.
13. Resuspend the cells in fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine and bring the volume up to 100 mL in the spinner flask. Continue to culture the cells in suspension with agitation on a magnetic stirring platform, inside a humidified incubator with 5% CO<sub>2</sub> at 37 °C.
14. The culture may reach 40–60 x 10<sup>6</sup> cells/mL between day 11–14.
15. Harvest cells when the desired cell number is achieved and proceed to downstream application, e.g., analysis of cells.

## General guideline for T cell culture in Xuri™ Cell Expansion System W25

The Xuri™ Cellbag™ bioreactors are single-use, functionally closed bags that provide a suitable environment for cell expansion while minimizing the risk of cross contamination.

The cells are generally inoculated into the Xuri™ Cellbag™ at the beginning of exponential expansion phase (e.g., day 5 after T cell activation). It is recommended to inoculate at minimum 150 x 10<sup>6</sup> viable cells into the Xuri™ Cellbag™ at the density of 0.5 x 10<sup>6</sup> viable cells per mL medium for further expansion.

1. Perform T cell activation and initial expansion phase in static culture platforms such as T-flask, G-Rex® or gas permeable cell-culture bags as described above, with the aim to

obtain a minimum 150 x 10<sup>6</sup> viable cells on day 5 after activation.

2. On day 5, determine total viable cell number and cell viability using standard cell counting protocols. Calculate how much fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine is needed to achieve the target cell density of 0.5 x 10<sup>6</sup> viable cells/mL in the Xuri™ Cellbag™ bioreactor.
3. Prepare the Xuri™ Cell Expansion System W25 as recommended by the supplier and set up the appropriate condition (e.g., CO<sub>2</sub>: 5.0%; Speed: 8 RPM; Angle: 6°; Temperature: 37 °C).
4. Add fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine into the Xuri™ Cellbag™ bioreactor and let the medium to acclimate inside the bioreactor as recommended.
5. Inoculate the cells into the Xuri™ Cellbag™ bioreactor using standard aseptic technique and start cell expansion.
6. Monitor cell growth daily using standard cell counting protocols. Add fresh TheraPEAK® T-VIVO® Cell Culture Medium with cytokine to re-adjust the cell density to 0.5 x 10<sup>6</sup> viable cells/mL until the maximum volume is reached (e.g., 1 liter in a 2L Cellbag™).
7. Monitor cell metabolism by measuring metabolites (e.g., Glucose, L-glutamine, Lactate and Ammonium) and cell growth conditions within the Xuri™ Cellbag™ bioreactor such as dissolved oxygen (DO) level and pH.
8. Start medium perfusion after the culture volume reaches 1L in the Cellbag™, using either shot perfusion or continuous perfusion mode as desired. The optimal cell expansion process may be established empirically by testing process parameters such as medium perfusion volume and rate, rocking speed and angle.

For example, medium perfusion volume may increase from 500 mL → 750 mL → 1000 mL → 1250 mL → 1500 mL over every 24 hours.

A medium bag containing sufficient TheraPEAK® T-VIVO® Cell Culture Medium with cytokine can be connected to the Xuri™ Cellbag™ and stored at room temp for up to one week.

When in use, minimize exposure of the medium to light.

9. The culture may reach 40–60 x 10<sup>6</sup> cells/mL between day 6–8 in the Xuri™ Cellbag™ bioreactor.
10. Harvest the cells when the desired cell number is achieved and proceed to downstream application, e.g., analysis of cells.

## IV. General recommendations

### Maintain good dissolved oxygen (DO) level

It has been observed that T cell proliferation *in vitro* is faster when the dissolved oxygen level is high. When using the TheraPEAK® T-VIVO® Cell Culture Medium, it is recommended to adopt processes that maintain good dissolved oxygen level during the cell expansion processes.

1. For optimal gas exchange in static T-flasks, it is recommended that medium depth be less than 3 mm.
2. For optimal gas exchange in static gas permeable cell culture bags, it is recommended that medium depth be less than 1 cm.
3. In G-Rex®, the cells reside on a gas permeable silicone membrane with very high oxygen transfer rate and the medium height does not hinder gas exchange. Therefore, there are no necessary changes to the culture conditions recommended by Wilson-Wolf.
4. In bioreactors, such as Cocoon®, Xuri™ Cellbag™, or stir-tank bioreactor, it is recommended to monitor the dissolved oxygen level and adopt processes that maintain the dissolved oxygen level above 80%.

### Optimal timing for Nucleofection® or electroporation

T cell activation via CD3 and CD28 using TransAct™, ImmunoCult™ or Dynabead™ leads to rapid cell proliferation which places great demands on the cellular machinery.

Nucleofection® or other electroporation procedures can efficiently introduce molecules into the cells, and are popular methods for genetic engineering in T cells. However, these membrane-

disrupting processes can negatively impact cell health. To repair the plasma membrane the cells need to uptake or synthesize membrane lipid molecules. In the absence of serum and serum-substitutes, this process can be slower than in serum-containing media.

The combination of cell activation and Nucleofection® Processes may result in greater cellular stress. It is recommended that the recovery time between cell activation and Nucleofection® in the TheraPEAK® T-VIVO® Cell Culture Medium be extended by 1 to 2 extra days to allow for better cell recovery. In the event that multiple Nucleofection® Processes are required for a particular T cell engineering protocol, further temporal separation of the events may be necessary.

## **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](http://www.lonza.com/legal), <https://www.lonza.com/about-us/strategy/intellectual-property>, and [www.lonza.com/privacy](http://www.lonza.com/privacy).

© 2026 Lonza. All rights reserved. CT-OP001 01/26.