A Novel Chemically Defined Medium Supports Superior Cross-platform T-cell Expansion

Lonza
Cell & Gene

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Abstract

One of the weak links in the current good manufacturing process of genetically engineered T cells for adoptive cell therapy is the use of human serum. Human serum and derived components are expensive, potentially contain adventitious agents requiring stringent safety testing and may contain factors detrimental for T-cell expansion. In addition, the performance of human serum may vary considerably from lot-to-lot, necessitating screening and stockpiling. The supply of high-quality human serum may be insufficient to meet global demand in the near future as more blockbuster adoptive T cell therapies are approved and become one pillar for modern medicine.

We have developed a cell culture medium, which incorporates only recombinant proteins, is free of undefined animal origin components, and requires only the addition of cytokines and activation agents, thus streamlining the CAR-T cell therapy manufacturing process. This medium supports superior T-cell expansion compared to other commercially available T-cell expansion media which require supplementation with human serum. T-cell manufacturing processes utilizing this chemically defined medium represent an important step forward in making adoptive T-cell therapy more consistent and scalable to better serve patients.

The absence of human serum and human plasma-derived proteins during the activation, transfection or transduction, and expansion processes necessitates some changes in common cell culture procedures. Alternative handling practices to be considered when using a chemically defined medium for CAR-T cell therapies will be discussed.

Methods

Medium preparation: Recombinant human IL-2 (R&D Systems) is added to the T-cell expansion medium at 100 IU/mL in all experiments. Human AB serum (Gemini) is used at 5% as indicated.

T-cell activation: Cryopreserved PBMCs or CD3+ T cells from healthy donors are thawed and seeded in 24-well plates (1.0 x 10^6 PBMCs or 0.5 x 10^6 CD3+ T cells) in 1mL medium. T Cell TransAct™ (Miltenyi Biotec) is used to activate T cells (10 µL/mL medium). On day 3, T Cell TransAct is removed by centrifugation.

T-cell expansion in T-flask: The cells are counted every 2–3 days. Fresh medium (with 100 IU/mL IL-2) is added to adjust the cell density back to about 0.5 x 10^6 cells/mL at the time of medium addition.

T-cell expansion in Cocoon® Platform, Xuri™ and Spinner flask: see details in each section.

Bench-scale T-cell Expansion in T-Flask or G-Rex®

T-cell expansion in T-flask using various medium with or without human AB serum

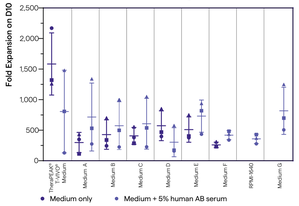
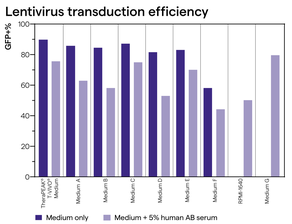


Figure 1. TheraPEAK® T-VIVO® Medium outperforms competitor media when measuring T cell fold expansion on day 10 (Mean±SD; n=3 donors, each donor is represented by the ●■▲ symbols).

Lentivirus transduction efficiency



T cells composition (CD4% and CD8%)

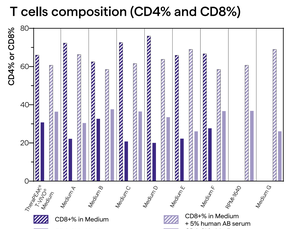


Figure 2. Lentivirus transduction efficiency and cellular phenotype is analyzed on day 9 after culturing with TheraPEAK® T-VIVO® Medium or other media in G-Rex® (Wilson Wolf, 10cm²). T cells are transduced with a lentivirus expressing GFP at MOI = 10 and analyzed via flow cytometry. Most of the cells are αβ T cells.

T-cell Expansion in Cocoon® Platform

Day	T-cell expansion process in Cocoon® Platform
3	TransAct™ removal. Begin medium recirculation
4–5	50% media exchange
6–9	75% media exchange

Day	TheraPEAK® T-VIVO® Medium	Medium A with 5% human serum
4	5.64 x 10^6 (95.8%)	6.53 x 10^6 (97.6%)
7	1.56 x 10^6 (89.8%)	1.56 x 10^6 (93.7%)
9	2.07 x 10^6 (96.7%)	2.34 x 10^6 (86.4%)

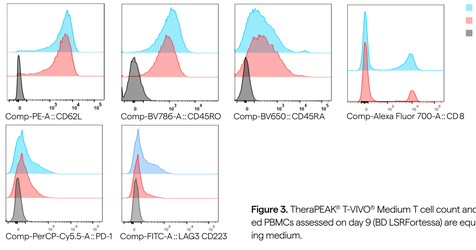
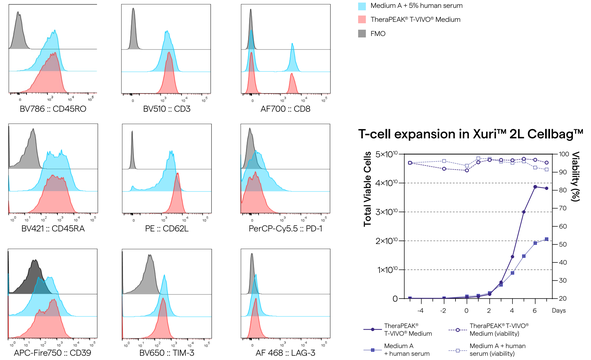


Figure 3. TheraPEAK® T-VIVO® Medium T cell count and phenotype from activated PBMCs assessed on day 9 (BD LSRFortessa) are equivalent to serum-containing medium.

Xuri™ Cell Expansion System W25

Day	T-cell expansion in Xuri™ Cellbag™
0	Inoculate cells into Xuri™ 2 L Cellbag™ @ 0.5 x 10^6 cells/mL
1	Add fresh medium to dilute back to 0.5 x 10^6 cells/mL
2	Add fresh medium to 1 L max volume
3–7	Perfusion

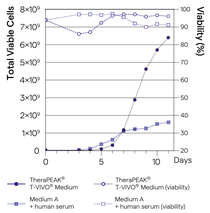


T-cell expansion in Xuri™ 2L Cellbag™

Figure 4. T-cell expansion from PBMCs (110 x 10^6 cells) by T Cell TransAct™ using TheraPEAK® T-VIVO® Medium and Medium A plus 5% human AB serum is tracked by daily cell count. On day 10, the expression of various cellular markers are analyzed by flow cytometry. Most of the cells are αβ T cells.

T-cell Expansion in Spinner Flask

T-cell expansion in 125 mL spinner flask



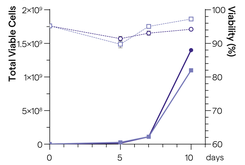
Day	T-cell expansion process in spinner flask
3–5	Add fresh medium to dilute back to 0.5 x 10^6 cells/mL, until reach max volume (100 mL)
6–11	Daily medium exchange (100 mL)

Figure 5. T cells in TheraPEAK® T-VIVO® Medium reach >60.0 x 10^6 cells/mL in 125mL spinner flask.

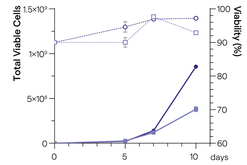
High DO Supports Optimal Cell Growth and Viability

	High DO	Low DO
Medium height	2 mm	6 mm

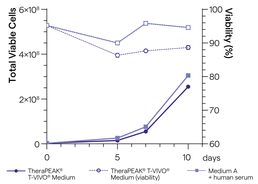
High DO, start with 1e6 PBMCs



High DO, start with 0.5e6 CD3 T cells



Low DO, start with 2e6 PBMCs



Low DO, start with 2e6 CD3 T cells

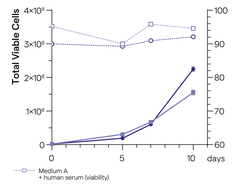


Figure 6. TheraPEAK® T-VIVO® Medium maintains >90% cell viability under optimal DO conditions.

Discussion

Summary: TheraPEAK® T-VIVO® Medium performs well in several cell culture platforms. Optimal performance is recorded when parameters promote, 1) high dissolved oxygen (DO) levels; 2) minimized presence of lysosomal enzymes from dying cells; and 3) minimized cellular abrasion from motion-based platforms. Users of the TheraPEAK® T-VIVO® Medium are encouraged to closely monitor the cell expansion process to achieve peak performance.

Chemically defined serum-free medium that delivers superior cell expansion will help the cell therapy industry via increase process control and simplify ensuring regulatory compliance. It will also enable the development of cell therapies that will present lower risk to patients and remove the variability associated with human-sourced components. Chemically defined TheraPEAK® T-VIVO® Medium represents a significant step forward in this direction.

Learn more:

All TheraPEAK® Products are produced according to applicable GMP standards and follow the USP/EP guidance for cell and gene therapy raw materials. It is the end user's responsibility to ensure full compliance with all regulations based on their use of Lonza's products in their specific process. TheraPEAK® Media Products are produced at FDA registered manufacturing sites with an ISO 13485 certified quality management system. This product is not for human or animal in vivo use, including use as a diluent or as an excipient, or for diagnostic use. This product is for use in GMP manufacturing processes or research use only. All trademarks belong to Lonza, registered in USA, EU or CH or to third party owners and are used only for informational purposes. The information contained herein is believed to be correct. No warranty is made, either expressed or implied. For more details: www.lonza.com/legal.

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