

# Superior T Cell Expansion Using a Chemically-defined Medium for Cell Therapy

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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 is expensive, potentially contains adventitious agents requiring stringent safety testing, may contain factors harmful for T cell expansion and necessitates screening and stockpiling as the quality varies considerably from lot to lot. The supply of high-quality human serum may be insufficient to meet future demand as more blockbuster adoptive T cell therapies are approved and become one pillar for modern medicine.

TheraPEAK® T-VIVO® Medium uses chemically-defined non-animal origin components, incorporates only recombinant human proteins and requires only the addition of cytokines and activation agents, streamlining the CAR-T cell therapy manufacturing process. TheraPEAK® T-VIVO® Medium supports superior T cell expansion compared to other commercial T cell expansion media supplemented with human serum on various platforms. T cell manufacturing processes utilizing chemically-defined TheraPEAK® T-VIVO® Medium represents an important step forward in making adoptive T cell therapy more consistent 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 are detailed here.

## Methods

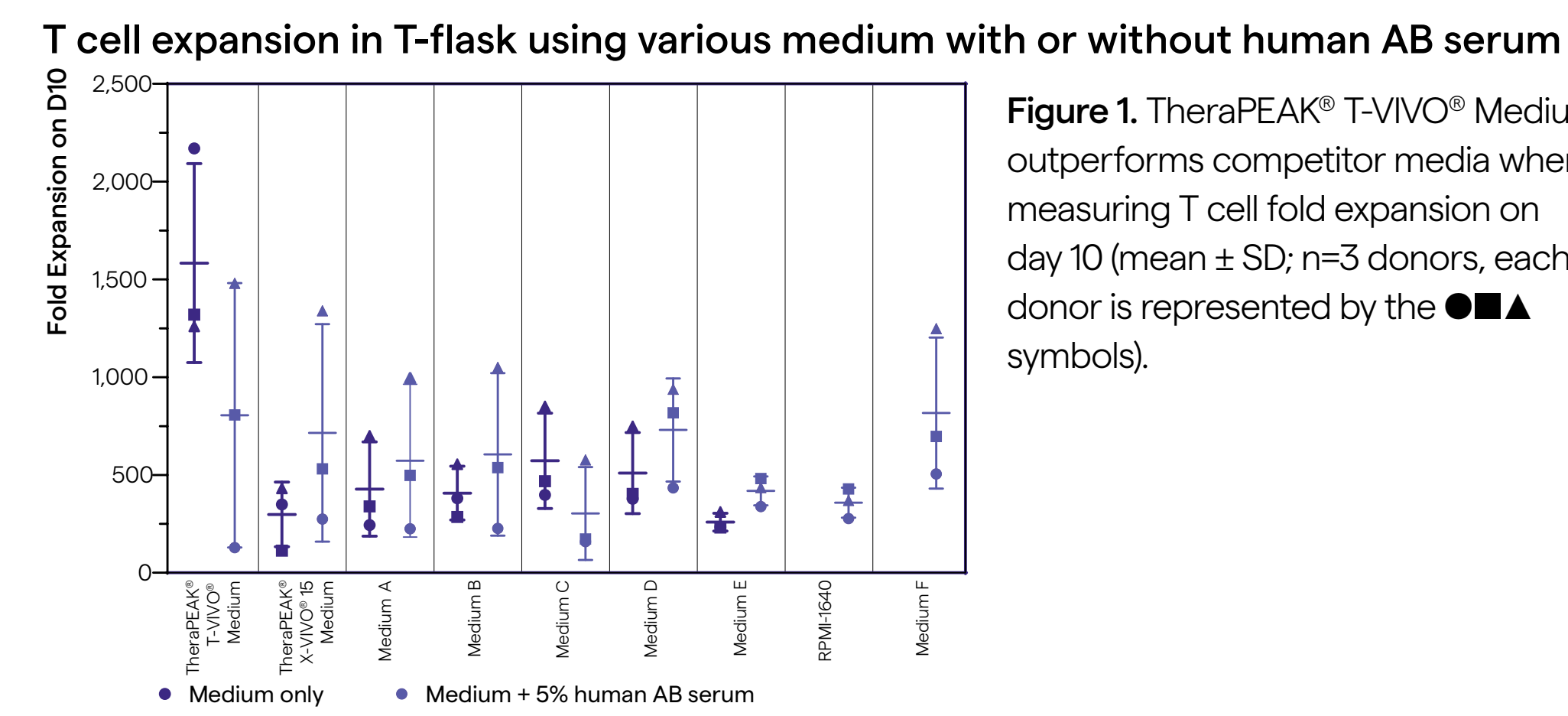
**Medium preparation:** Recombinant human IL-2 (R&D Systems) is added to the T cell expansion medium at 100 IU/mL in all experiments. Human AB serum (Gemini) is used at 5% as indicated.

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

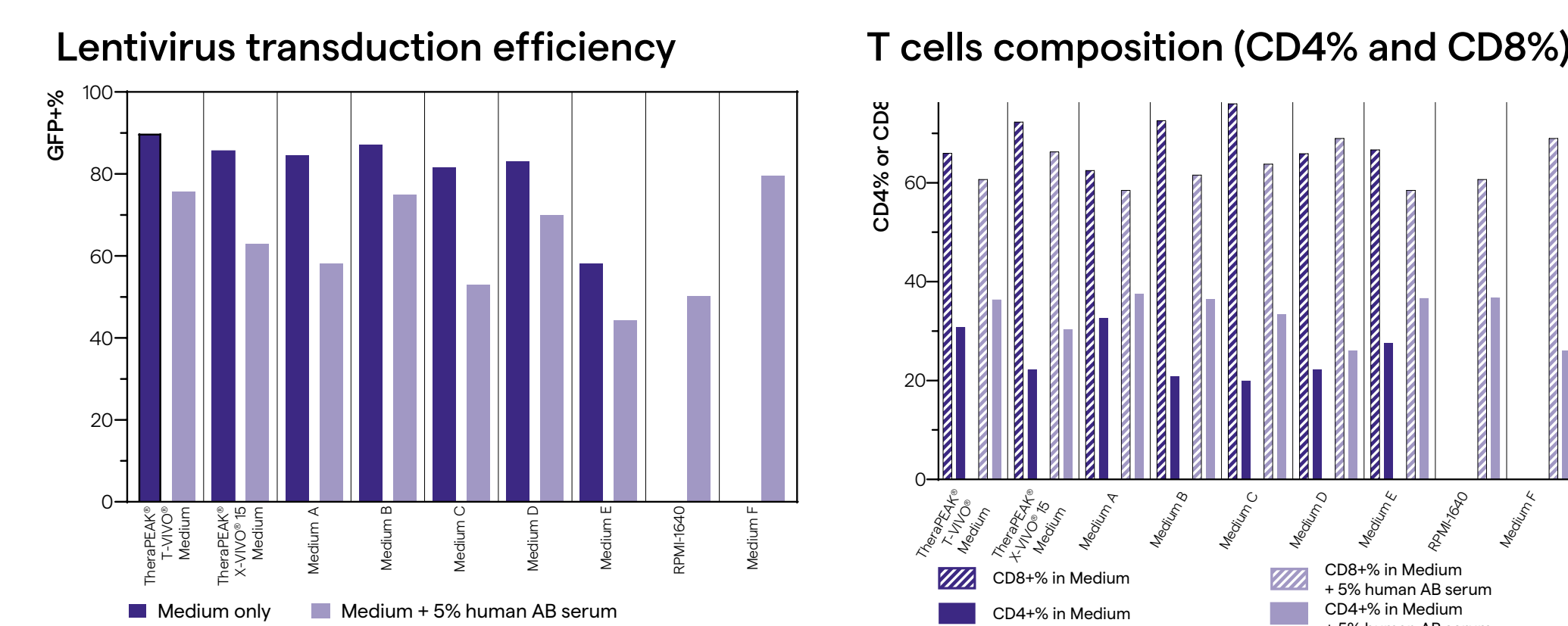
**T cell expansion in T-flask:** The cells are counted every 2–3 days. Fresh medium (with 100 IU/mL IL-2) is added to adjust the cell density back to about 0.5 x 10<sup>6</sup> cells/mL at the time of medium addition.

**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



**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).



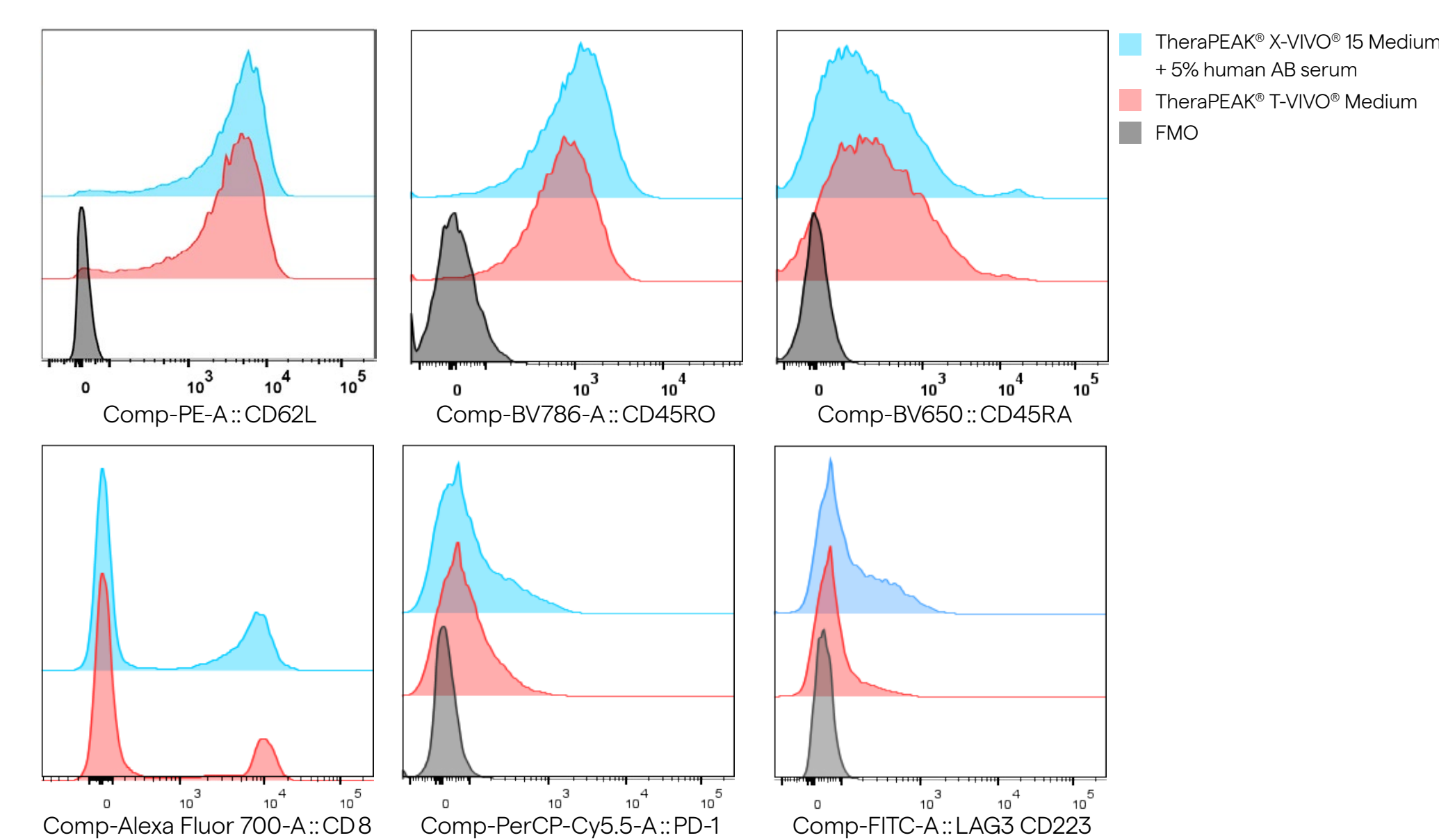
**Figure 2.** Favorable transduction efficiency and cellular phenotype was measured on day 9 after culture with TheraPEAK® T-VIVO® Medium in a G-Rex (Wilson Wolf, 10 cm<sup>2</sup>). T cells were transduced with a lentivirus expressing GFP at MOI = 10. Analysis via flow cytometry (BD FACSCelesta).

## 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	

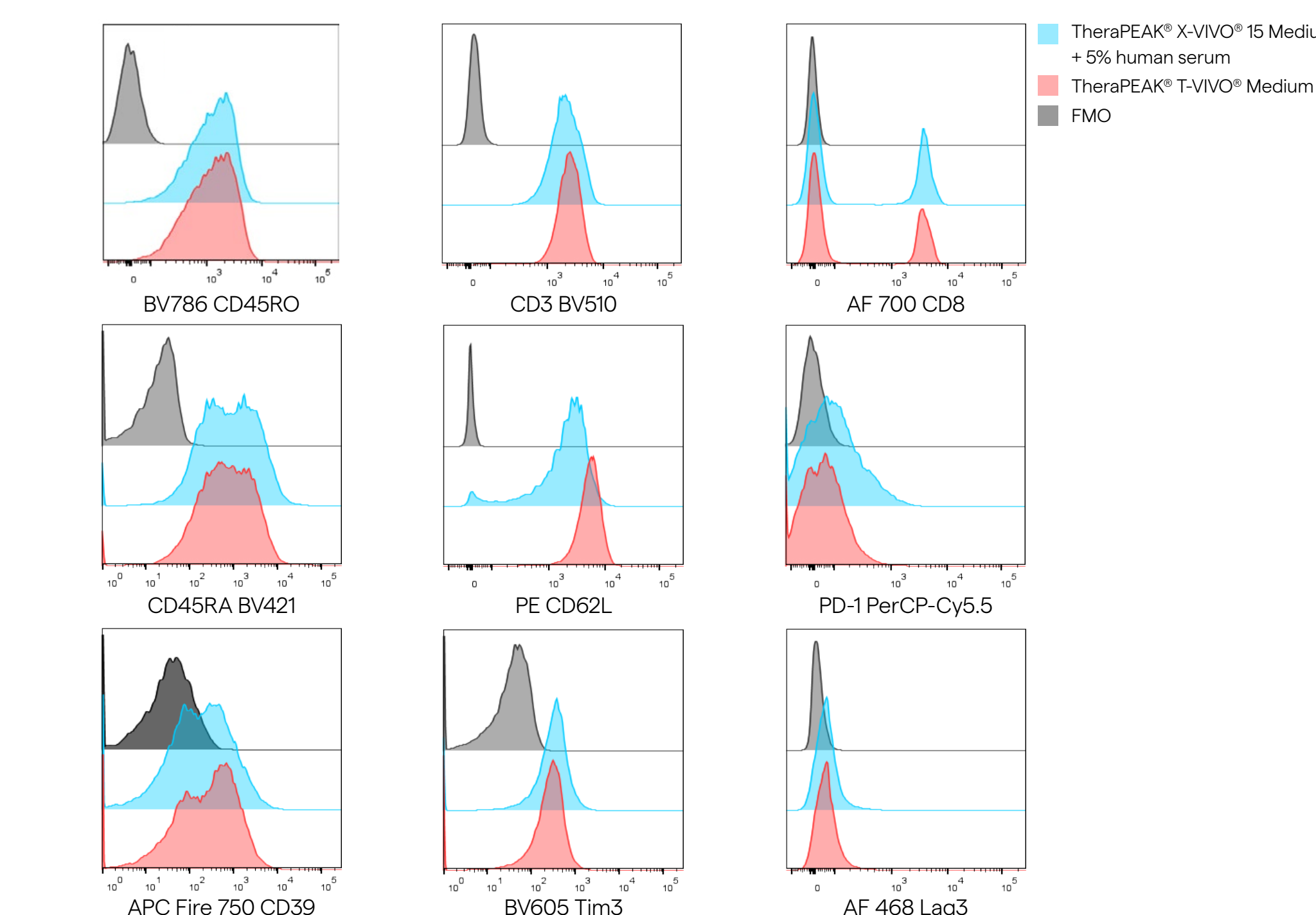
Day	TheraPEAK® T-VIVO® Medium	TheraPEAK® X-VIVO® 15 Medium + 5% human serum
4	5.64 x 10 <sup>8</sup> (95.8%)	6.53 x 10 <sup>8</sup> (97.6%)
7	1.56 x 10 <sup>9</sup> (89.8%)	1.56 x 10 <sup>9</sup> (93.7%)
9	2.07 x 10 <sup>9</sup> (96.7%)	2.34 x 10 <sup>9</sup> (86.4%)



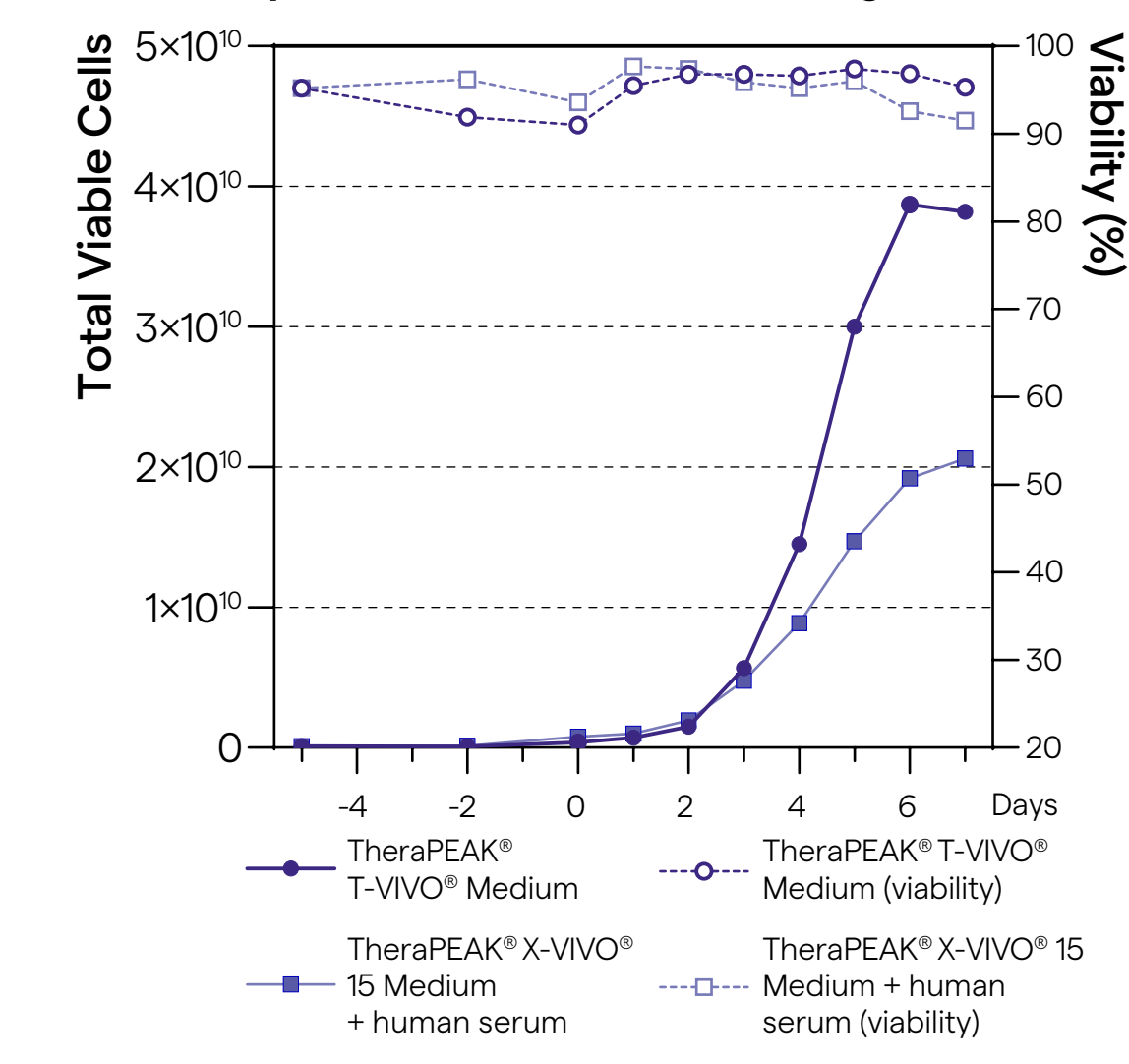
**Figure 3.** TheraPEAK® T-VIVO® Medium T cell count and phenotype from activated PBMCs assessed on day 9 (BD LSRFortessa) are equivalent to serum-containing medium.

## Xuri Cell Expansion System W25

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



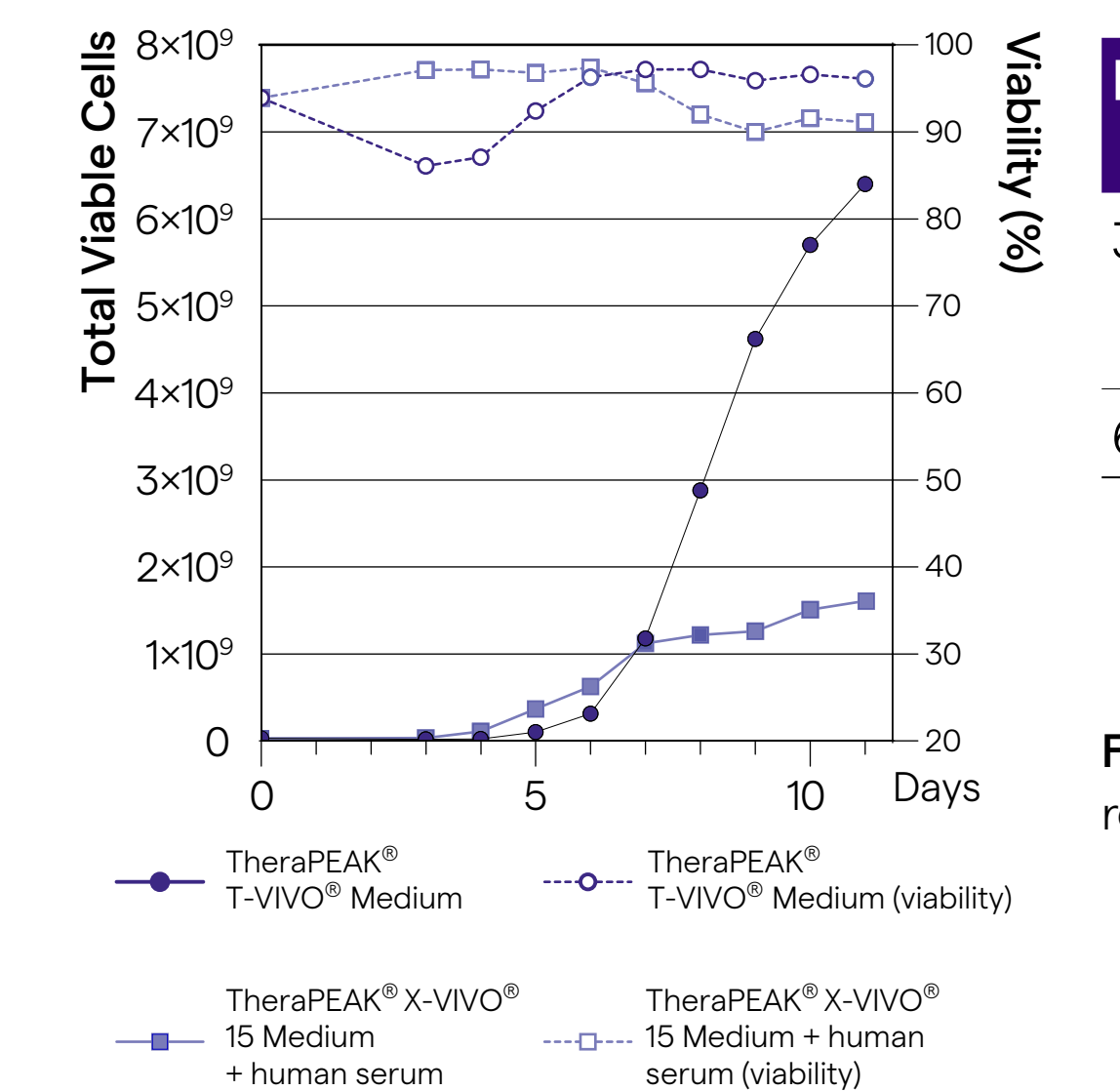
## T cell expansion in Xuri 2L Cellbag



**Figure 4.** T cell expansion in TheraPEAK® T-VIVO® Medium and TheraPEAK® X-VIVO® 15 Medium plus 5% human AB serum (Gemini) is tracked by daily cell count. On day 10, cell samples are analyzed by flow cytometry (BD FACSCelesta).

## T Cell Expansion in Spinner Flask

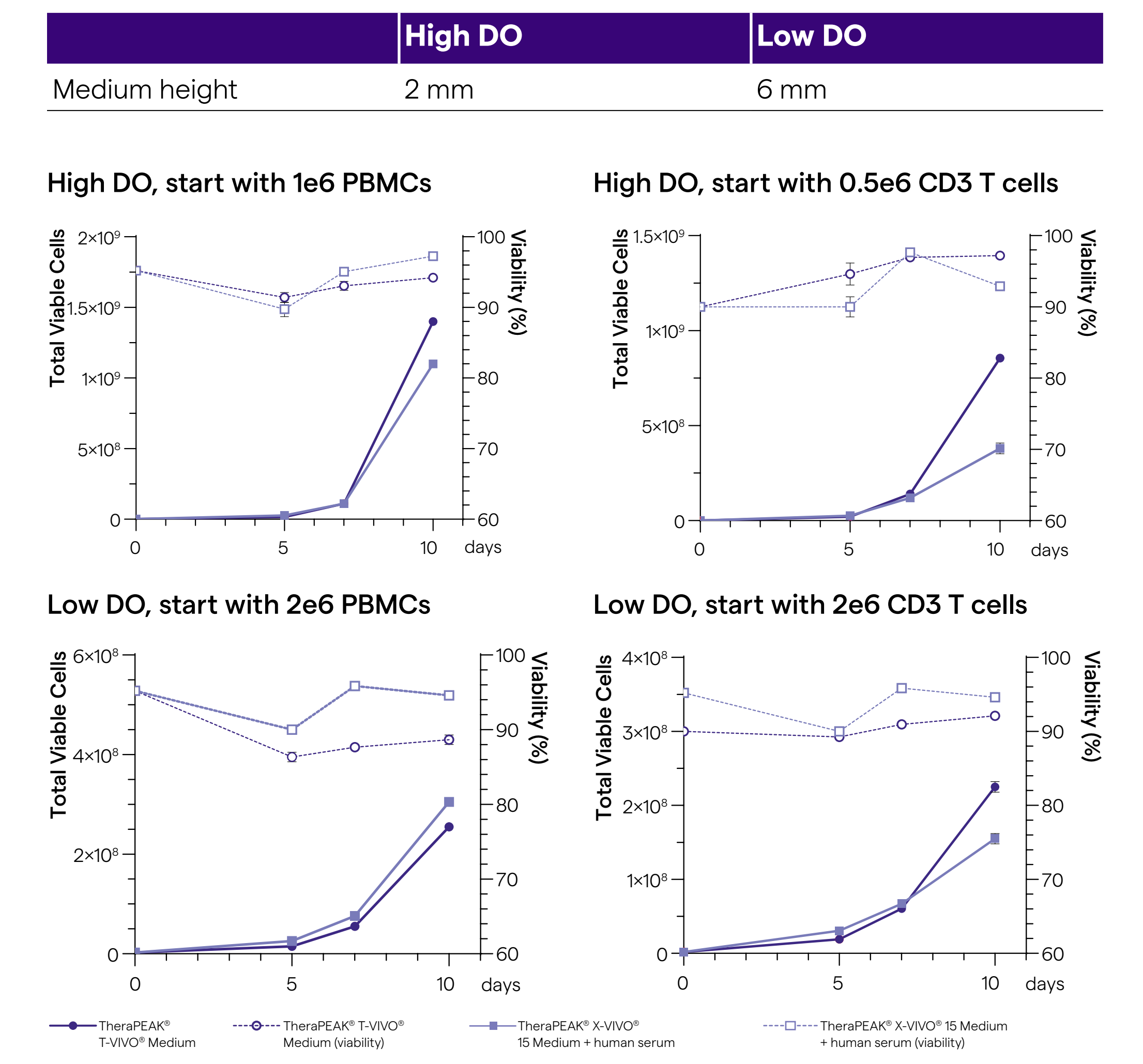
### T cell expansion in 125mL spinner flask



Day	T cell expansion process in spinner flask
3–5	Add fresh medium to dilute back to 0.5 x 10 <sup>6</sup> 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 a peak density of >55.0 x 10<sup>6</sup> cells/mL.

## High DO Supports Optimal Cell Growth and Viability



**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.

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