

Determining Optimal Seeding Density for Human T Cell Expansion in G-Rex

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Introduction

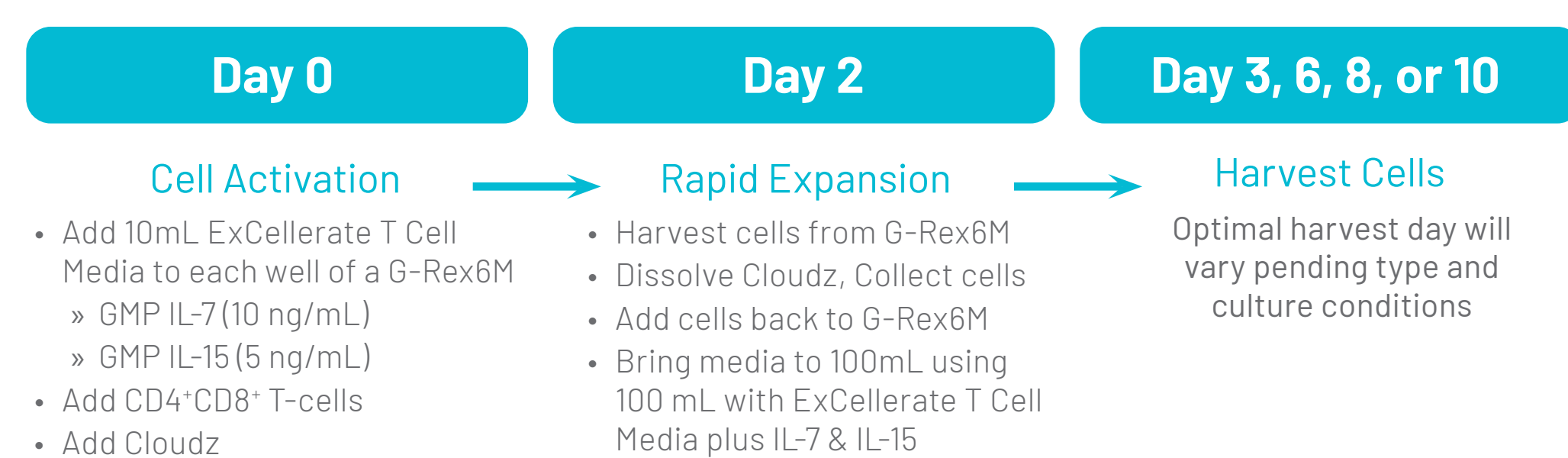
Efficiently expanding human T cells in a closed-system while maintaining a consistent cell phenotype is an active challenge for autologous and allogeneic T cell therapy manufacturers. Cell phenotype drift and variability in cell yield are common pain-points when transitioning from research protocols (i.e. flask or well plates) into larger scale, closed manufacturing systems (i.e. gas permeable bags, stirred tank bioreactors). This variability can be partially attributed to the introduction of motion and liquid media exchanges to accommodate cell expansion within larger volumes. G-Rex bioreactors have been shown (e.g. TIL, NK cells, T cells) to resolve these challenges by providing a static growth environment for cells, void of motion or media exchange, and promoting consistent cell expansion with reproducible cell phenotypes.

Optimizing cell expansion in G-Rex at small-scale is key to achieving efficient cell expansion. In this study we investigated the kinetics of human T cell expansion in small scale G-Rex6M bioreactors using ScaleReady reagents. Specifically, we determine the affect of seeding density on fold expansion and total cell yield. In addition, we look at the phenotypic composition of expanded T cells, including total CD4⁺ and CD8⁺ cells as well as memory CD4⁺ T cells.

Methods

Human CD3⁺ T cells from 3 donors seeded at two densities (0.5x10⁶, 1.0x10⁶) into G-Rex[®]6M Well Plates. For each donor, separate G-Rex6M Well Plates were seeded for undisturbed cell expansion until harvest and cell analysis at Day 0, 3, 6, 8, and 10. Triplicates for each donor were run at each collection timepoint. All cells were cultured using ExCellerate[™] T Cell Expansion Media supplemented with GMP rhIL-7 and GMP rhIL-15. Cloudz[™] T Cell Activation Kit was used for cell activation. Cloudz CD3/CD28 microspheres were added at Day 0 and removed at Day 2. After Cloudz removal, cell were returned to G-Rex 6M Well Plates for undisturbed rapid expansion using ExCellerate T Cell Expansion Media supplemented with IL-7 and IL-15. A detailed experimental procedure overview is depicted below.

Experimental Overview



Conditions

Condition	Cell Number	Cell Density	Cloudz Concentration	Checkpoint / Harvest Day	Glucose & Lactate Measurement
#1	5x10 ⁶	0.5x10 ⁶ /cm ²	25 µL/1x10 ⁶ cells	Day 3 Day 6 Day 7	Day 6 Day 7
#2	1x10 ⁷	1x10 ⁶ /cm ²	25 µL/1x10 ⁶ cells	Day 8 Day 10	Day 8 Day 9 Day 10

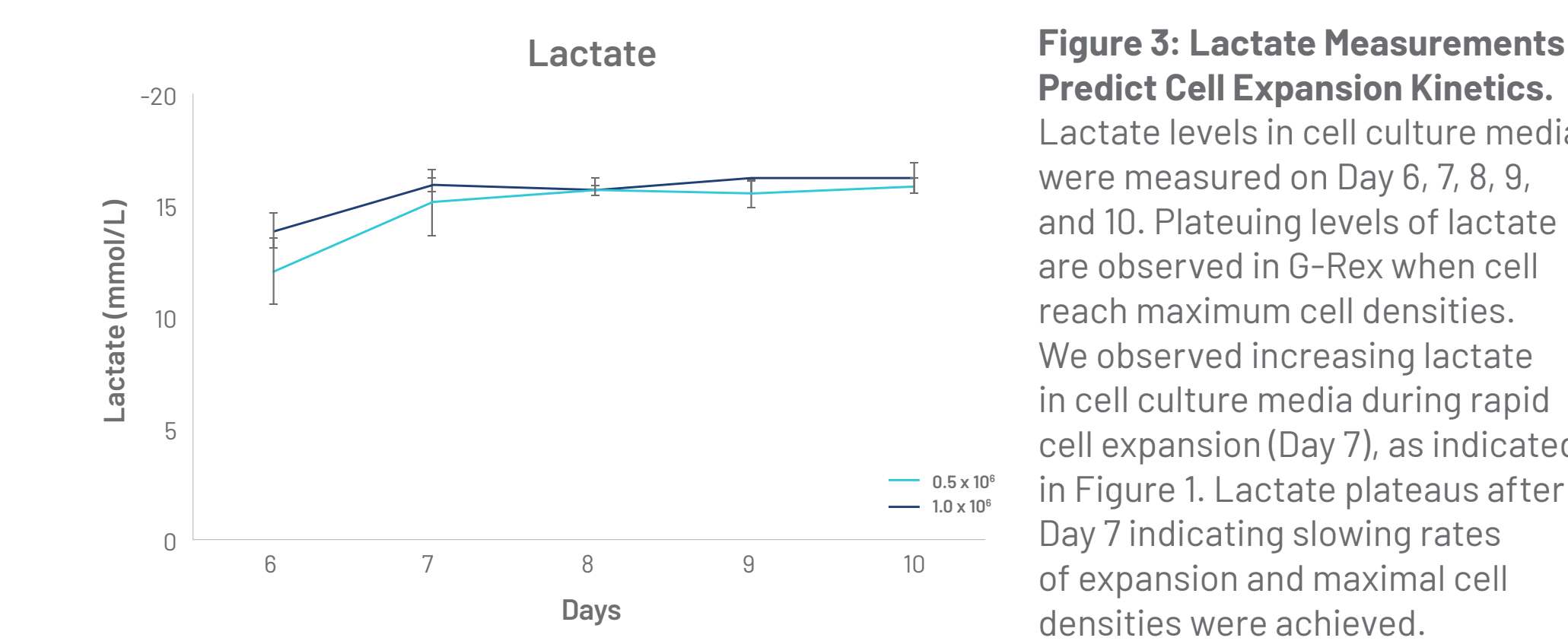
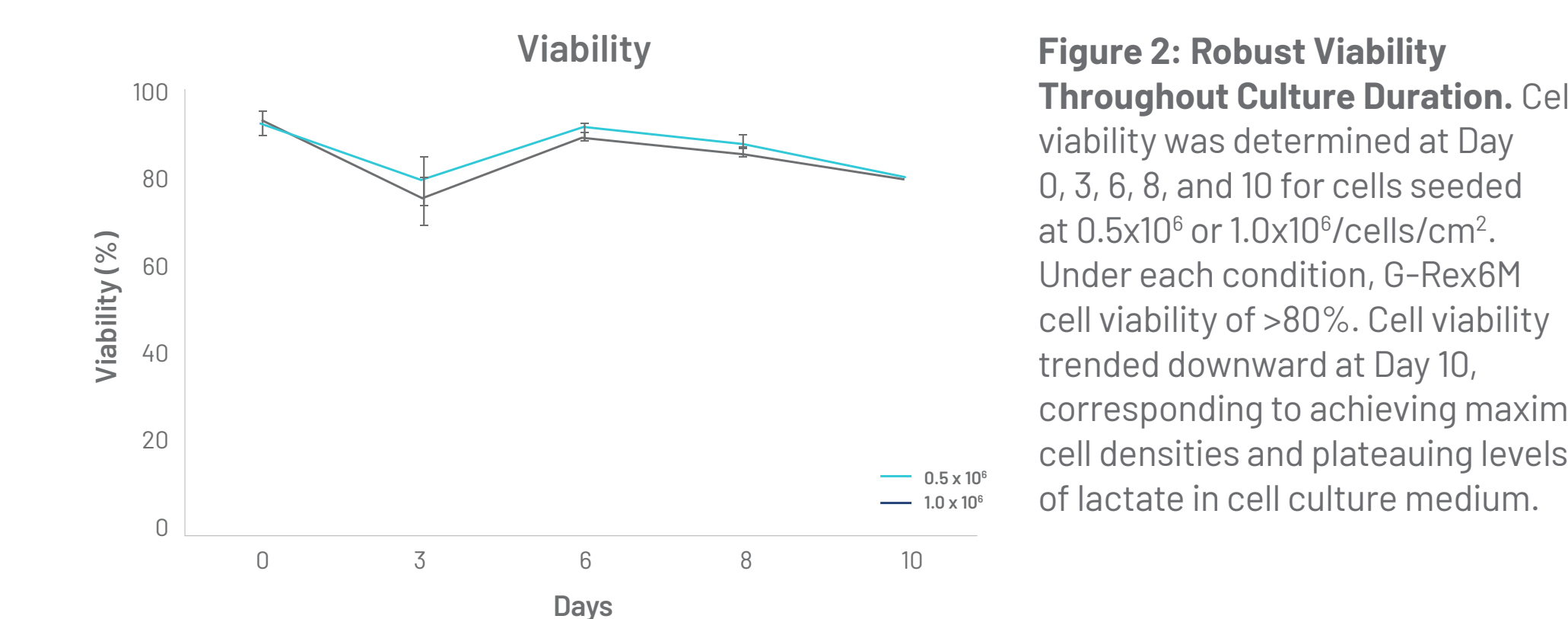
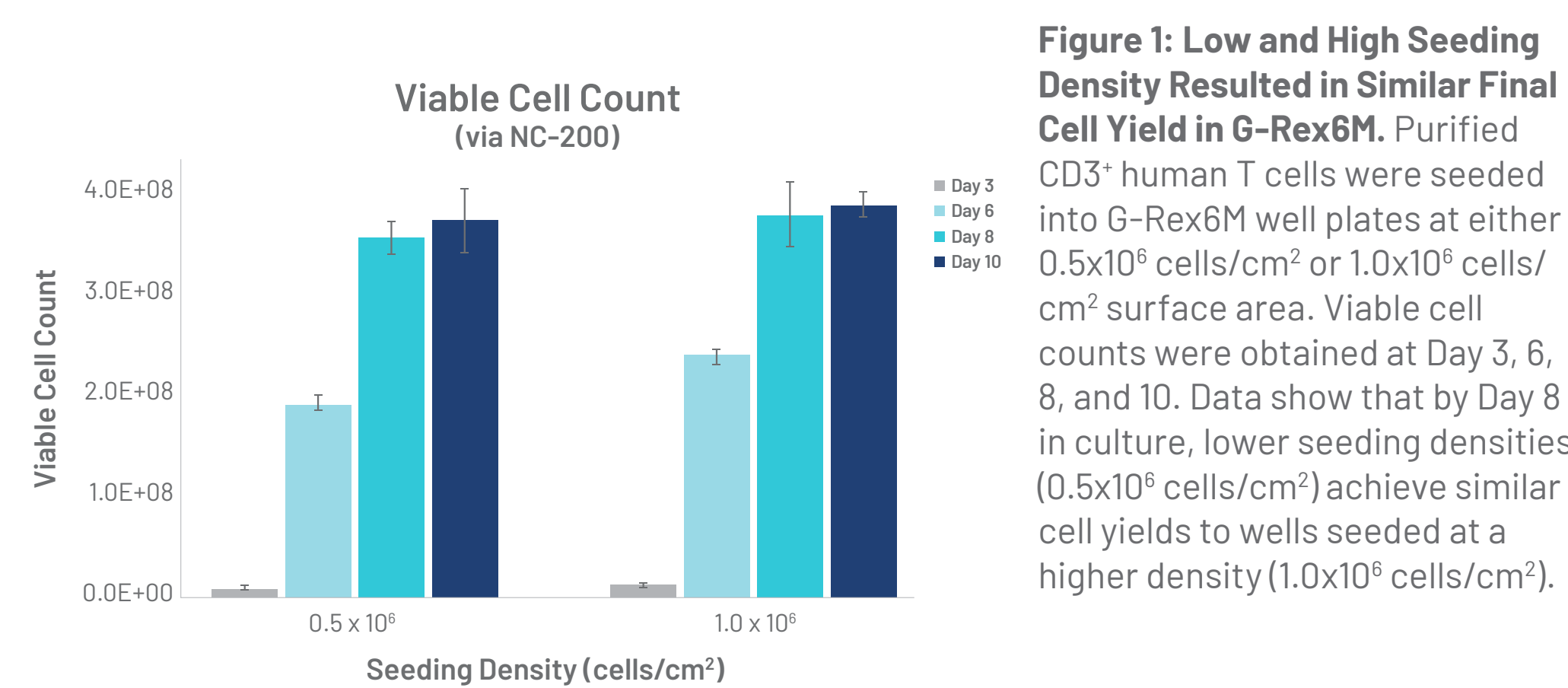
*Each condition was repeated with 3 donors

Raw Materials

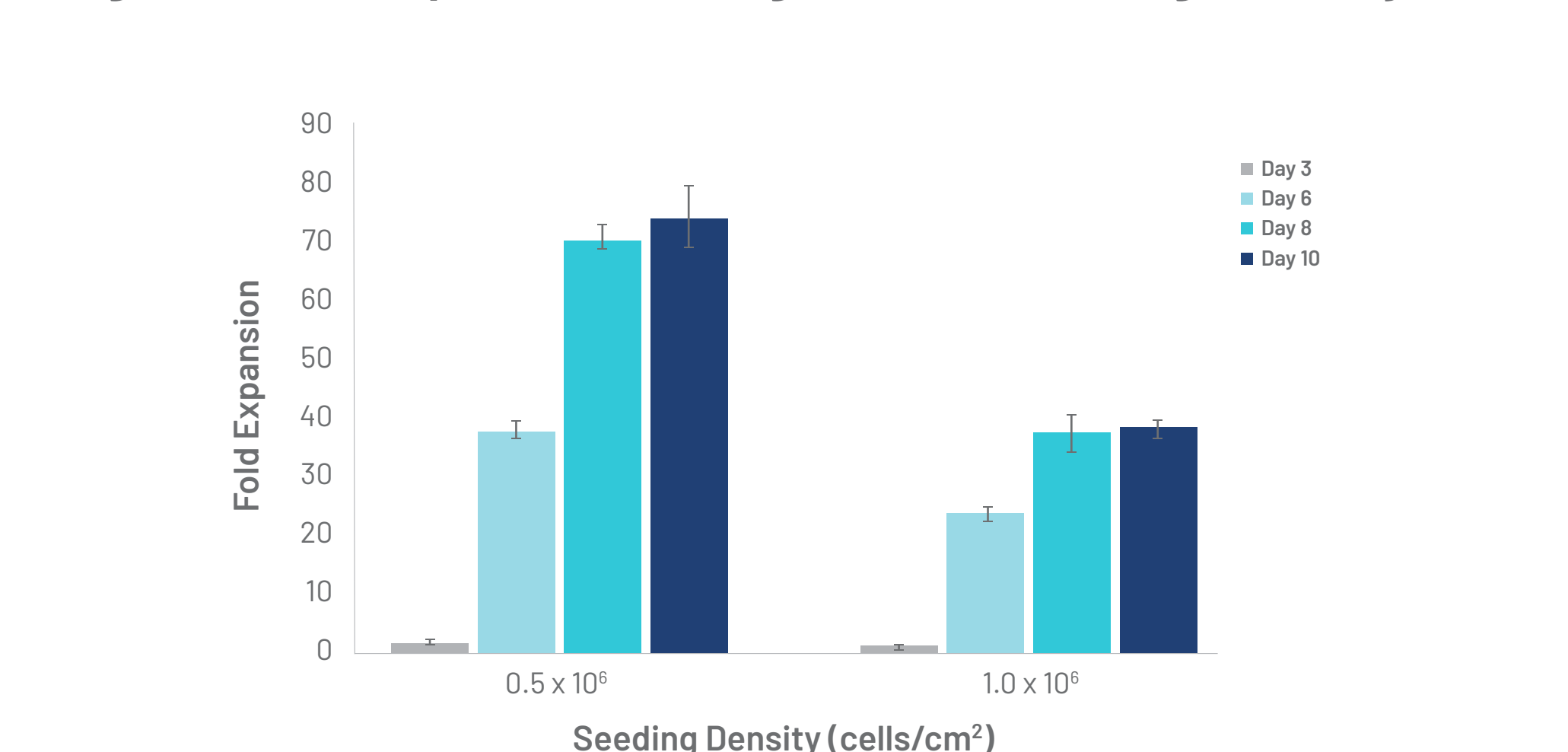
Product Name	Concentration	Catalog Number	Supplier
G-Rex [®] 6M Well Plate	N/A	80660M	Wilson Wolf
GMP rhIL-7	10 ng/mL	207-GMP	Bio-Techne
GMP rhIL-15	5 ng/mL	247-GMP	Bio-Techne
Cloudz [™] T Cell Activation Kit	25 µL/1x10 ⁶ cells	CLD001	Bio-Techne
ExCellerate [™] T Cell Media	100 mL/well	CCM030	Bio-Techne

Results

Similar Cell Expansion Kinetics and Cell Yields Across Seeding Densities

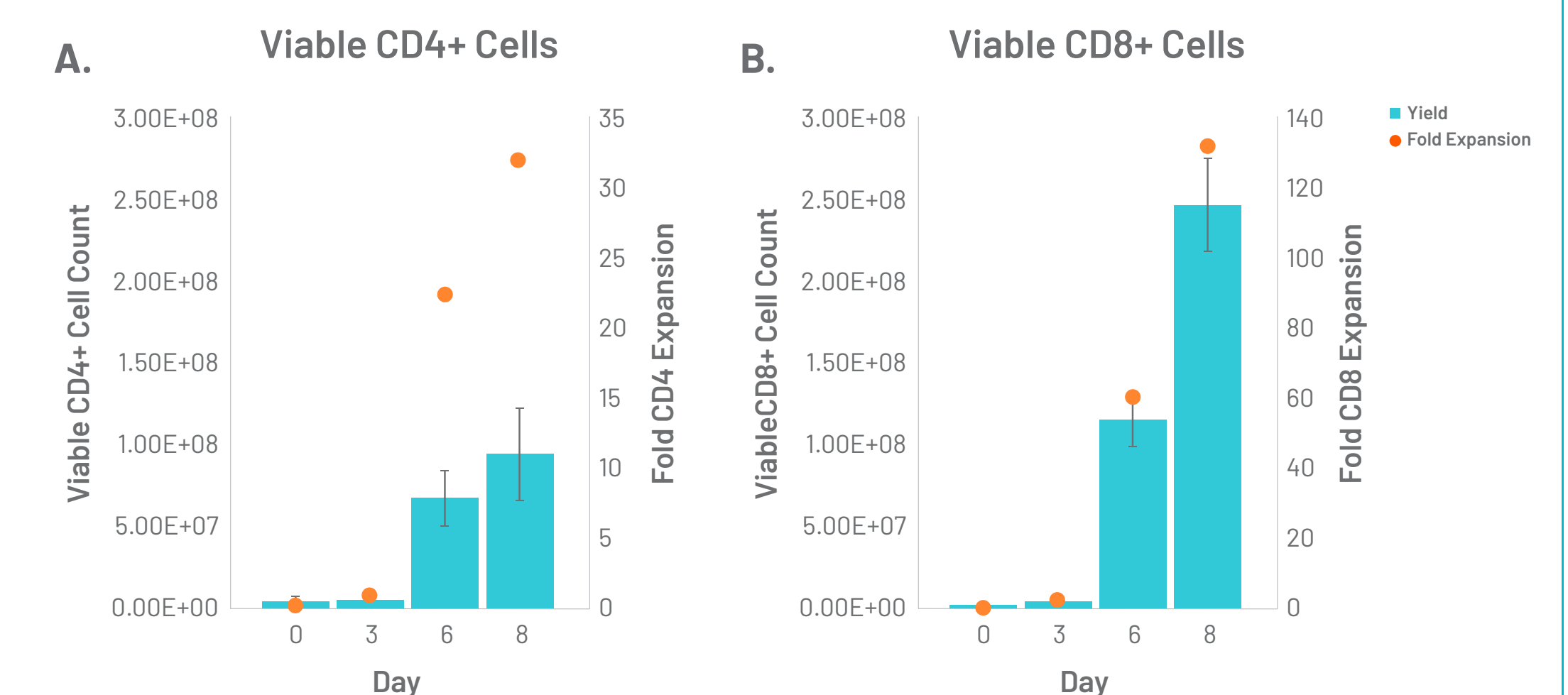


Higher Fold Expansion Using Lower Seeding Density

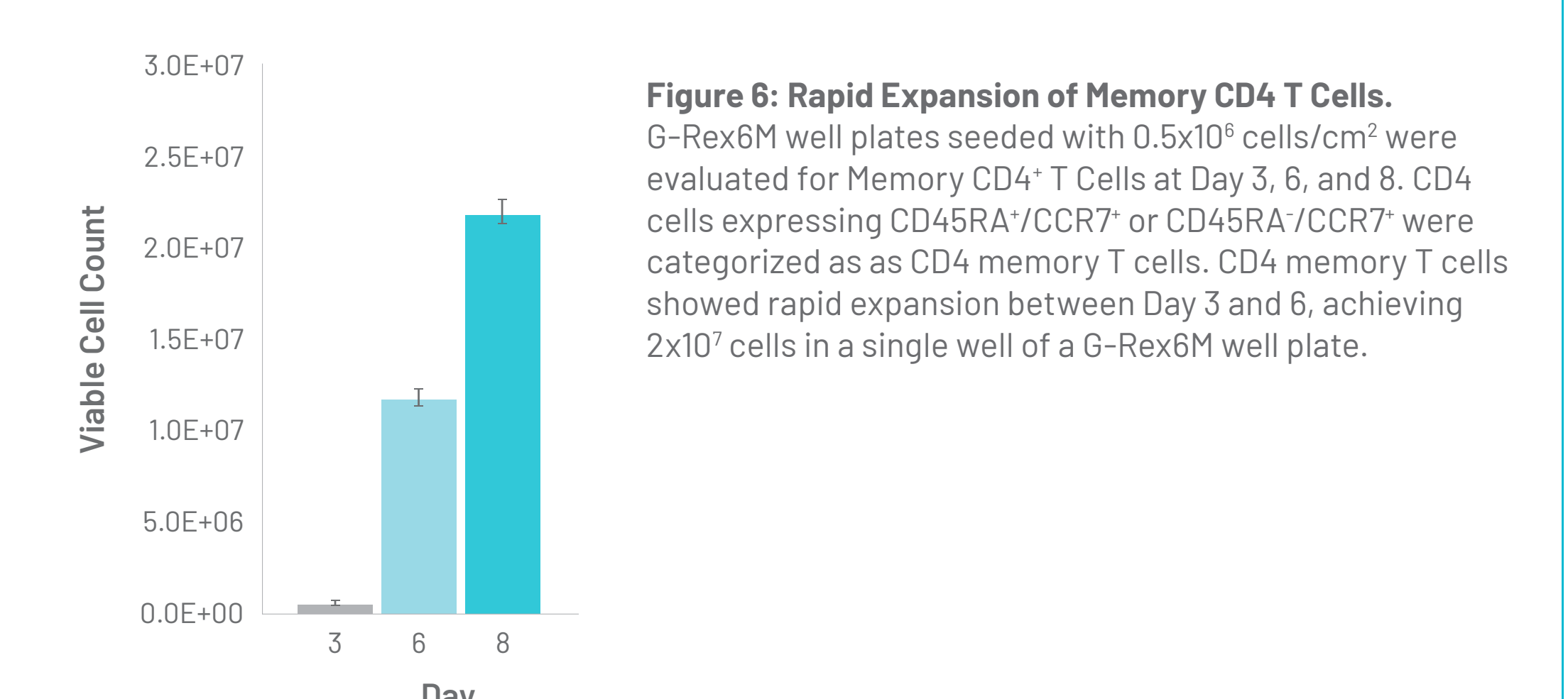


Results

High Fold Expansion of CD4⁺ and CD8⁺ Cells



Rapid Expansion of Memory CD4⁺ T Cell Population



Conclusion

- Maximum T cell yield and expansion kinetics can be achieved using a lower starting cell density in the G-Rex. This improves the efficiency of T cell expansion by minimizing the number of donor cells needed to achieve maximum expansion within a G-Rex.
- G-Rex facilitates rapid expansion of T cells
- G-Rex T cell expansion is compatible with ScaleReady reagents:
 - » Cloudz T Cell Activation Kit
 - » GMP IL-7 & GMP IL-15
 - » ExCellerate T Cell Expansion Media
- Small-scale preliminary data suggests:
 - » Successful expansion of pre-selected CD4⁺ and CD8⁺ cells in a G-Rex6M Well Plate
 - » Seeding densities of 0.5x10⁶ cells/cm² and 1x10⁶ cells/cm² produced 3.7x10⁸ and 3.8x10⁸ amount of total viable cells, respectively, with > 85% viability.
 - » Desired yield, phenotype, and composition of final product may dictate harvest date.

Contact us: info@scaleready.com

ScaleReady is a Joint Venture formed by Bio-Techne, Fresenius Kabi, and Wilson Wolf. Combining selected offerings from the three partners, the ScaleReady manufacturing platform combines tools and technologies for cell culture, cell activation and expansion, gene editing, and cell processing.

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