



# G-Rex® Fill-and-Forget Workflow

## Touch-free T Cell Expansion with Bio-Techne Cytokines & Media

Immune cell therapies require predictable and scalable cell manufacturing processes utilizing streamlined workflows to minimize variability and maximize efficiency.

G-Rex bioreactors from Wilson Wolf are designed to be used in a "fill-and-forget" manufacturing workflow with Bio-Techne reagents and cytokines. During T cell expansion, manual interventions for media change and cytokine supplementation can be eliminated. This reduces risk of contamination and greatly streamlines manufacturing workflows, while reducing reagent and overhead costs.

Here, we compare T cell expansion using the recommended fill-and-forget workflow with a traditional mid-point cytokine spike workflow. Cytokine concentrations are quantified at various timepoints throughout expansion using Ella™, the Simple Plex immunoassay platform. Cell health is evaluated via expansion, viability, and phenotype.

This application note demonstrates hands-free T cell expansion using Bio-Techne's GMP cytokines and media in the G-Rex bioreactor platform – zero touchpoints, and no cytokine spiking. Simply fill-and-forget.

### Key Takeaways

- Using G-Rex bioreactors and Bio-Techne reagents, cytokine supplementation and media changes can be eliminated from T cell manufacturing workflows.
- Cells grown using the fill-and-forget workflow showed equivalent or improved expansion, viability, and phenotypes compared to those grown using the mid-point cytokine spike workflow, indicating cytokine supplementation is not beneficial to culture kinetics.
- While cytokines were consumed over a 9-day growth period, the remaining concentrations were sufficient to promote robust cell expansion.



Unlike any other cell therapy manufacturing technology, G-Rex is automatic. Using Bio-Techne reagents, and a fill-and-forget workflow, G-Rex bioreactors, eliminate intervention and set the stage for high throughput assembly line based manufacturing. Furthermore, reagent costs are reduced, and operational flexibility is enhanced.

Contact ScaleReady for samples to try our recommended workflows.

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## Materials and Methods

Product	Material	Part #
G-Rex 6M plate*	Culture Vessel	80660M (ScaleReady)
GMP Human T Cell Media	Media	CCM038-GMP
GMP IL-2 (200 IU)**	Cytokine	BT-002-GMP
GMP IL-7 (10 ng)**	Cytokine	BT-007-GMP
GMP IL-15 (10 ng)**	Cytokine	BT-015-GMP
Ella	Analytical Instrument	600-100
IL-2 (72x1)	Simple Plex Cartridge	SPCKB-PS-000295
IL-7 (72x1)	Simple Plex Cartridge	SPCKB-PS-000506
IL-15 (72x1)	Simple Plex Cartridge	SPCKB-PS-000500

\* Available exclusively from ScaleReady

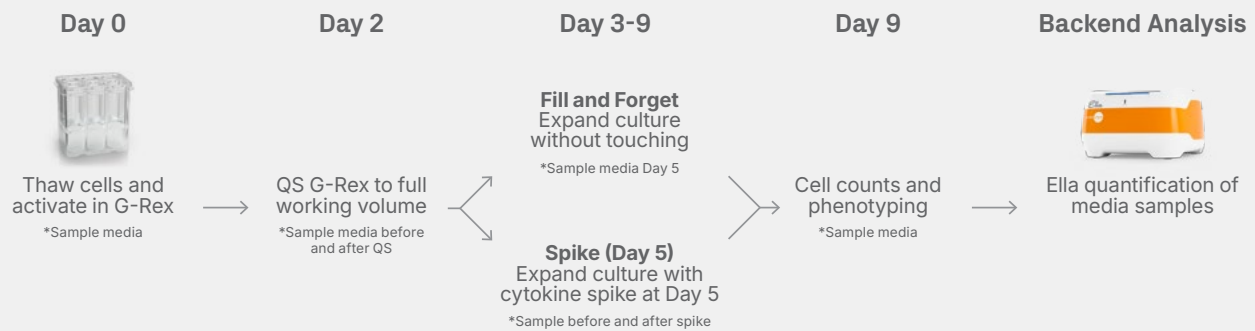
\*\* Available from ScaleReady

G-Rex is a registered trademark of Wilson Wolf Manufacturing, LLC

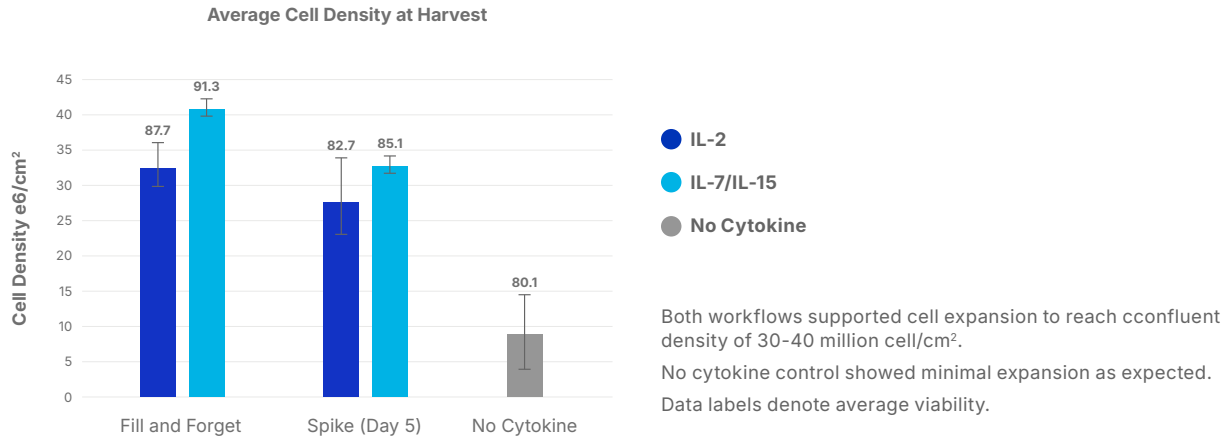
## Experimental Workflow

Isolated T cells were thawed, plated, and activated in a G-Rex 6M at 0.5e6/mL/cm<sup>2</sup> using GMP Human T Cell Media supplemented with 5% hAB serum and either 200 IU/mL IL-2 or 10 ng/mL IL-7 and 10 ng/mL IL-15. On Day 2, G-Rex was filled quantum satis (QS) with fresh media. Media supernatant was collected for measuring

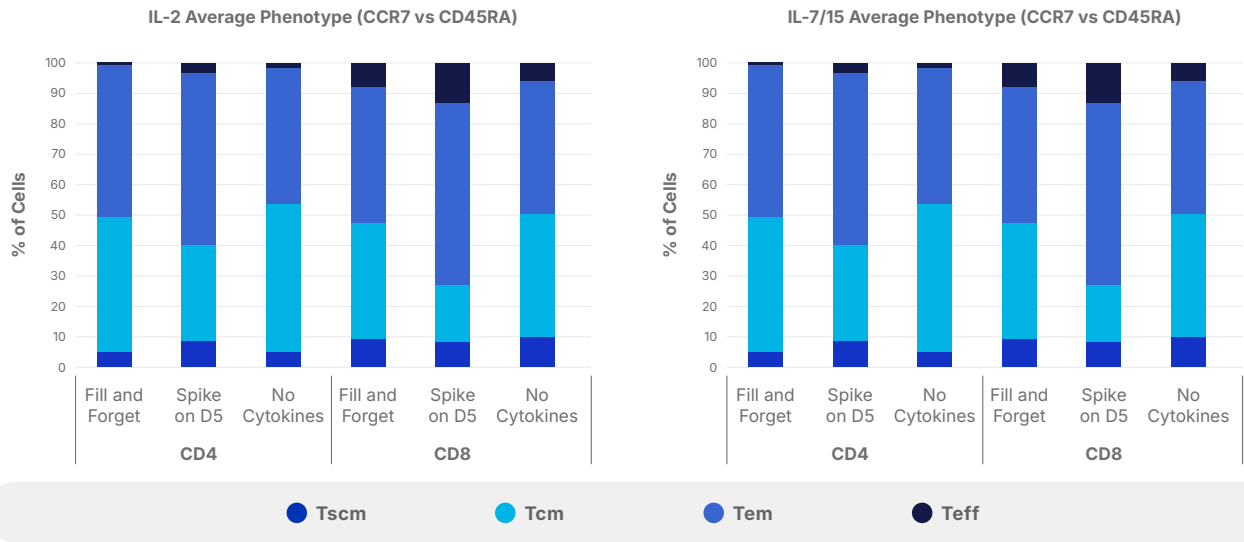
cytokine concentrations on days 0, 2 (pre- and post-QS), 5 (pre- and post-spike if applicable), and 9. Cytokine concentrations were quantified using the Ella Simple Plex automated immunoassay platform. All values shown were averaged across three donors.



## Fill-and-Forget Workflow Produced Equivalent T Cell Expansion and Viability



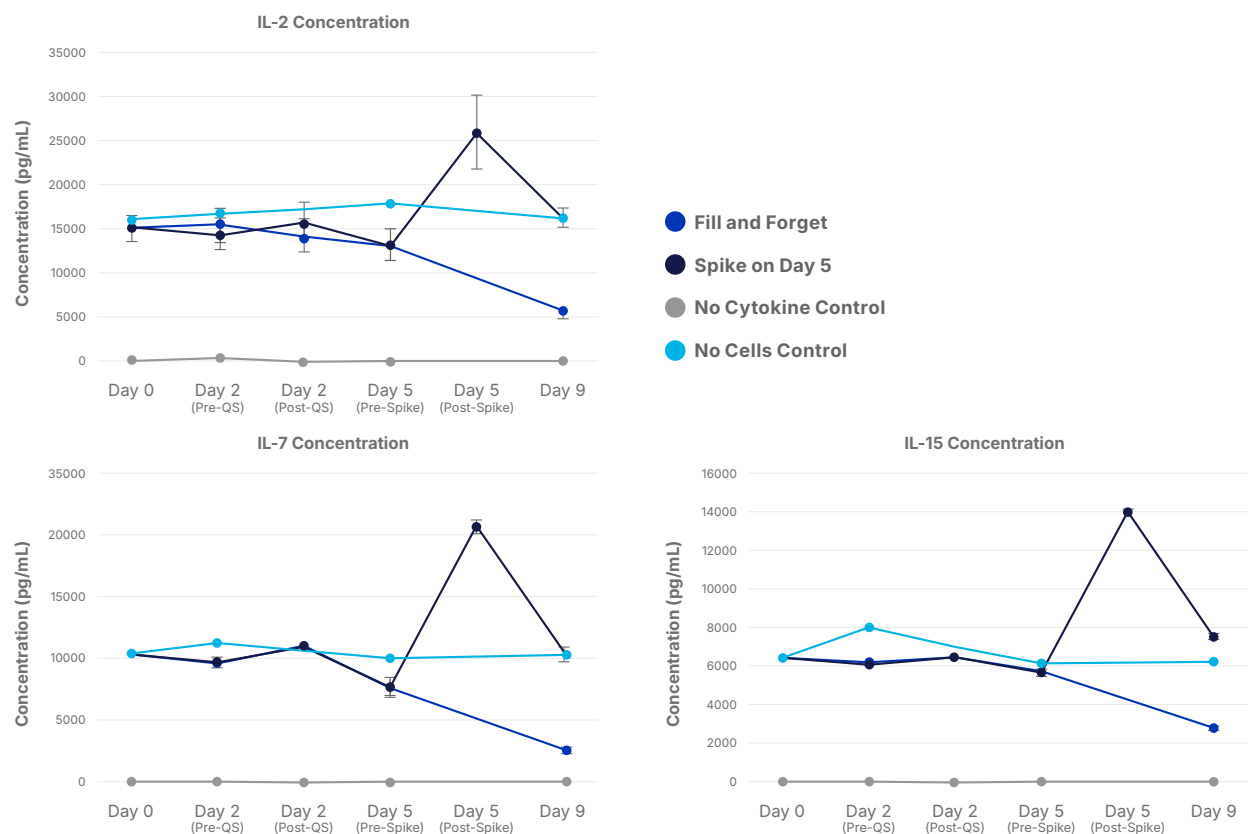
## Fill-and-Forget Workflow Produced Equivalent Expanded T Cell Phenotypes



Phenotype was assessed by flow cytometry using CCR7 and CD45RA to identify T cell memory populations.

Average memory phenotype was similar for both workflows.

## Fill-and-Forget Cytokine Concentrations were not Fully Consumed During Expansion



Over the 9-day culture period, we observed that the cytokine concentrations in the fill-and-forget workflow declined after culture day 5 as proliferative cells consumed the cytokines. Importantly, starting concentrations of 200 IU for IL-2 or 10 ng/mL for IL-7 and IL-15 were not fully consumed during the culture period.

Conversely, spiking cytokines midway through culture led to an excess of cytokines that was brought back to the starting concentration by the end of cell expansion.

No cells control (media in a G-Rex without any cells) showed cytokine concentrations did not diminish throughout culture period.

For the IL-2 lot used, 200 IU is equivalent to 17,860 pg/mL.

## Conclusion

Bio-Techne, in partnership with ScaleReady, provides solutions to streamline and simplify immune cell therapy manufacturing workflows. This application note demonstrates hands-free T cell expansion using Bio-Techne's GMP cytokines and media in the G-Rex bioreactor. Overall, this study shows that robust T cell expansion is easily achieved with a fill-and-forget workflow using Bio-Techne reagents and G-Rex

bioreactors. Elimination of interventions creates an automatic T cell manufacturing process. Ultimately, this automatic fill-and-forget workflow enables high throughput assembly line based cell therapy manufacturing, which will improve consistency, reduce reagent costs, and enable large-scale manufacturing, at low cost.

Learn More | [bio-techne.com/scaleready](https://bio-techne.com/scaleready)

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