



Application Note

Manufacturing CAR-T cells in chemically defined, serum-free TheraPEAK® T-VIVO® Cell Culture Medium on the Cocoon® Platform

Automated serum-free CAR-T manufacturing on the Cocoon® Platform

The production of Chimeric Antigen Receptor (CAR) engineered T cells involves critical steps for activating, delivering CAR, and expanding genetically modified T cells. This complex, labor-intensive process often requires significant operator interaction. The Cocoon® Platform is an automated manufacturing solution with enhanced process flexibility that enables integrated T cell selection, activation, transduction, expansion, and final product harvest within a functionally closed, single-use cassette.

Reliance on human serum may present challenges when manufacturing genetically engineered T cells. Specifically, current good manufacturing practice (cGMP) regulations require stringent quality standards when using human serum. Moreover, human serum and its derivatives are costly, exhibit inter-lot variability, and may contain adventitious agents that require rigorous safety testing.

In this application, we demonstrate the feasibility of manufacturing CAR-T cells in serum-free culture conditions in an automated process enabled by the Cocoon® Platform. Leveraging the Lonza TheraPEAK® T-VIVO® Cell Culture Medium (a chemically-defined, non-animal origin, serum-free medium), we generated robust CAR-T cells exhibiting strong phenotype and function in a 9-day process.



Serum-free manufacturing of CAR-T cells on the Cocoon® Platform

- Automated CAR-T cell manufacturing process enables optimal T-cell activation, transduction, and expansion in serum-free culture.
- Optimally-designed media exchange regimen drives robust CAR-T cell expansion and supports high cell viability.
- Notable CAR-T product function: strong potency and rapid anti-tumor response
- PBMC input material enables direct production of CAR-T cells without need for upstream T-cell selection.
- The Cocoon® Platform Serum-free CAR-T cell process is compatible with retroviral and lentiviral transduction, as well as non-viral gene transfer methods.

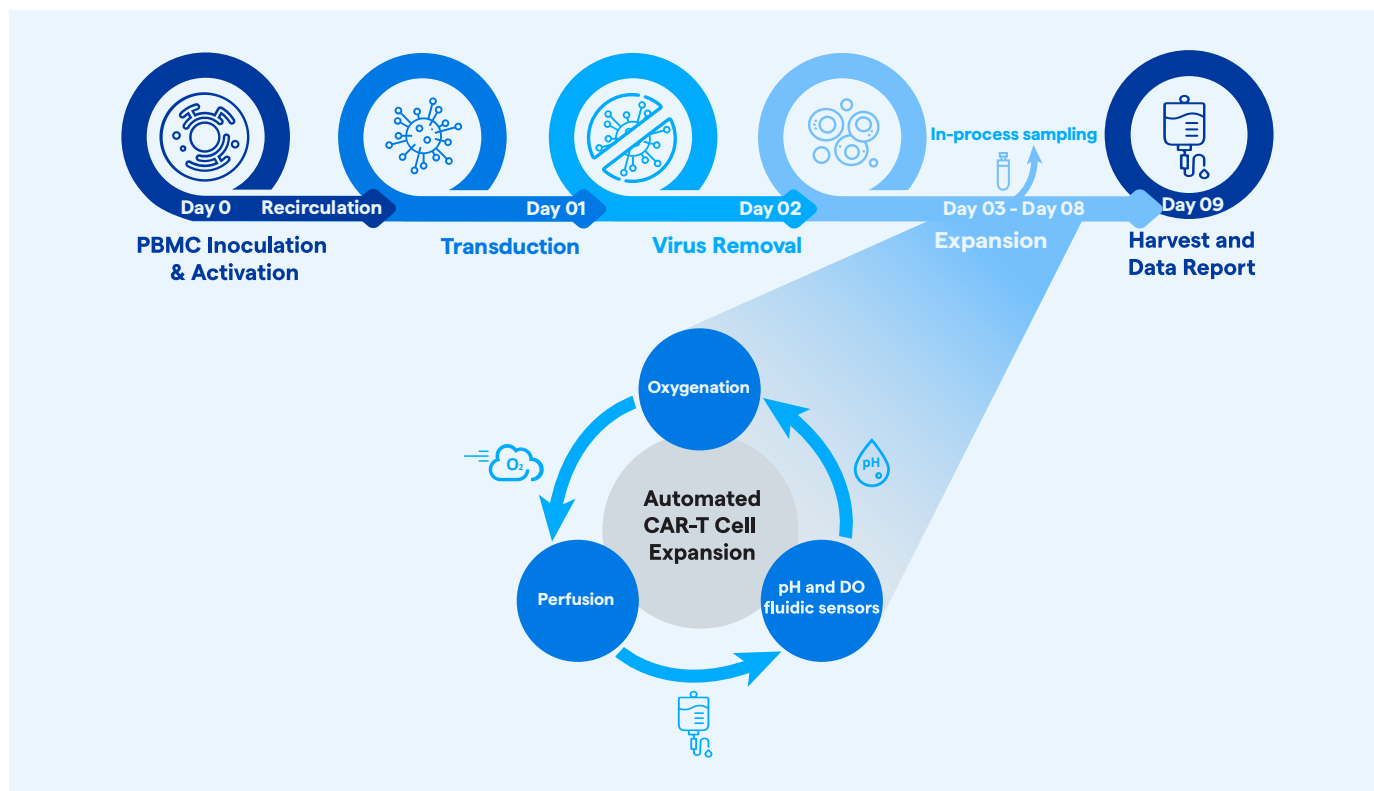


Figure 1: Cocoon® Platform process overview for manufacturing CAR-T cells in TheraPEAK® T-VIVO® Cell Culture Medium:

The Cocoon® Platform utilizes customizable, functionally closed, single-use cassettes. In this process, cryopreserved human peripheral blood mononuclear cells (PBMC) from healthy donors (n=3) are thawed and resuspended in the serum-free T-VIVO® media with T cell activating agents,

and are introduced into the Cocoon® Cassette. The PBMC are subsequently transduced for 24 hours, after which time virus vector is removed from culture and replaced with expansion medium. Programmable and automated media exchanges are performed from day 2 to day 8 with in-process sampling on day 6. On day 9, at the end of the process, the product is harvested. Electronic batch records and data logs are digitally exportable.

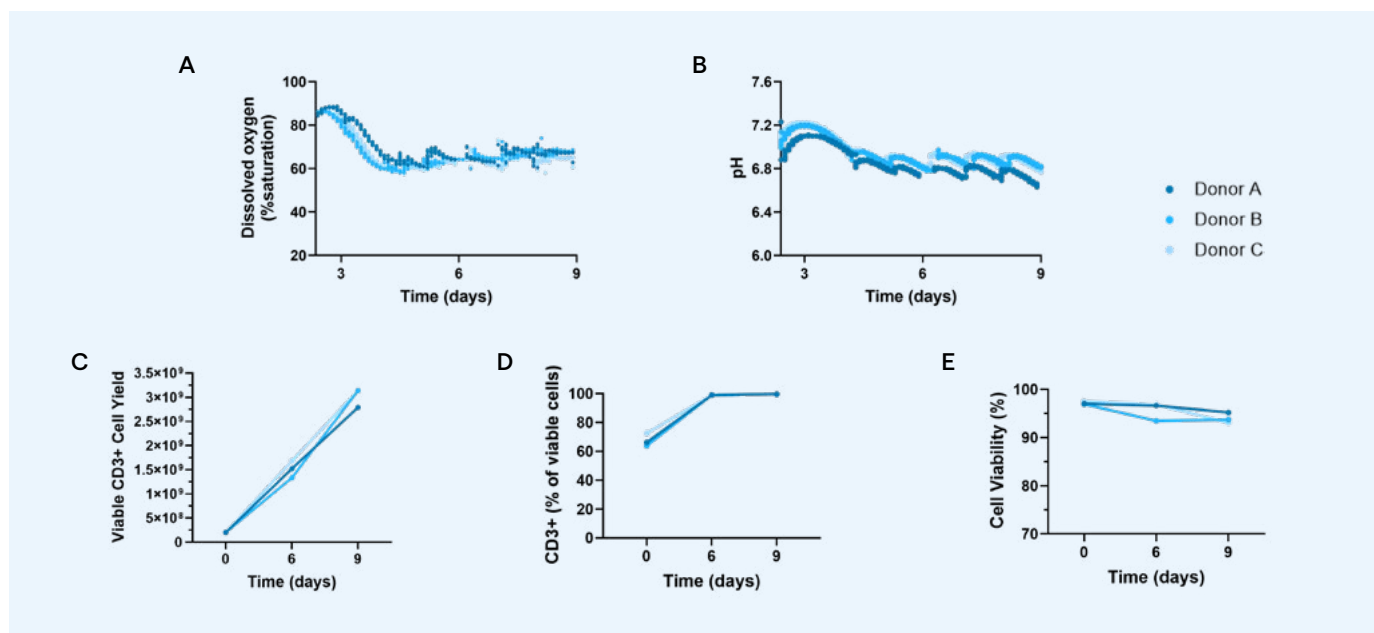


Figure 2: Cocoon® Platform CAR-T cell expansion:

Throughout the manufacturing process, the Cocoon® Platform performs real-time monitoring of temperature, environmental CO₂, pH, and culture dissolved oxygen levels to enable optimal culture conditions for T cell activation and expansion.

A, B) Dissolved oxygen, and pH measurements are captured continually at 10-minute intervals for the duration of the manufacturing process.

C) Viable T cell yields achieved for each production are measured at mid-process (on day 6, with an average of 1.62e9 cells) and at the end of process

(on day 9, with an average of 3.06e9 cells). Each manufacturing run was seeded with 2e8 PBMC on day 0. Consistent growth kinetics are observed for all three donors.

D) Preferential expansion of T cells is achieved, for each donor, in the serum-free Cocoon® Platform CAR-T manufacturing process.

E) Cell viability was assessed at the start of process (day 0), mid-process (day 6), and at harvest (day 9), using NC-200 NucleoCounter™, demonstrating maintenance of highly viable, healthy T cells throughout the manufacturing process.

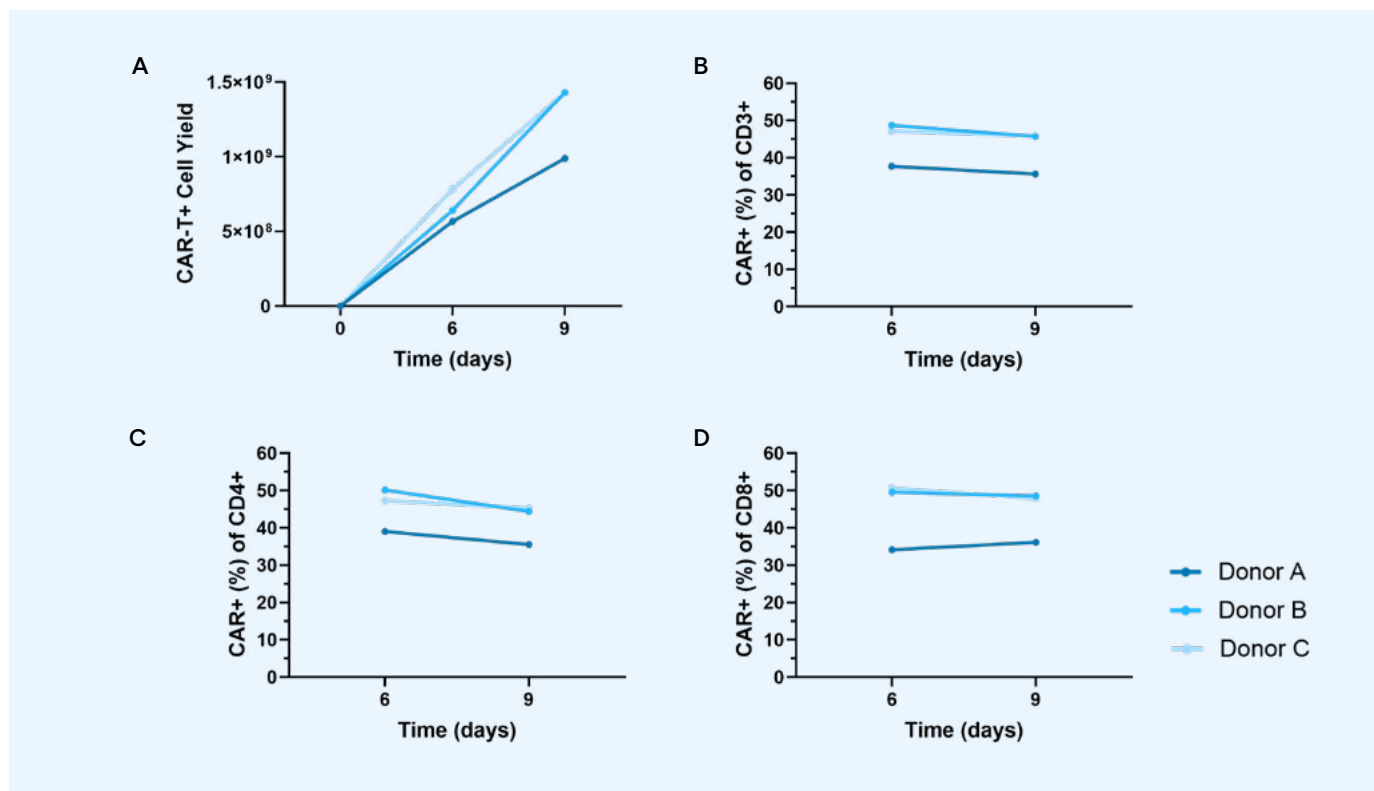


Figure 3: Cocoon® Platform manufactured CAR-T cell phenotypic characterization:

A) CAR-T cells expand well in the Cocoon® Platform using serum-free TheraPEAK® T-VIVO® Cell Culture Medium media, showing consistent expansion trends from days 6-9 of manufacture, irrespective of donor-to-donor variability.

B, C, D) T-cell transduction, and CAR expression over time are shown

for total CD3+ T cells (B), CD4+ T cells (C) and CD8+ T cells (D). On day 1, cells were transduced at an MOI of 0.5 using CD19-CAR lentivirus vector (manufactured at Lonza). Robust expression of CD19-CAR was observed for all three donors. Both CD4+ and CD8+ T cell subsets showed similar transduction and maintained robust growth profiles throughout the process.

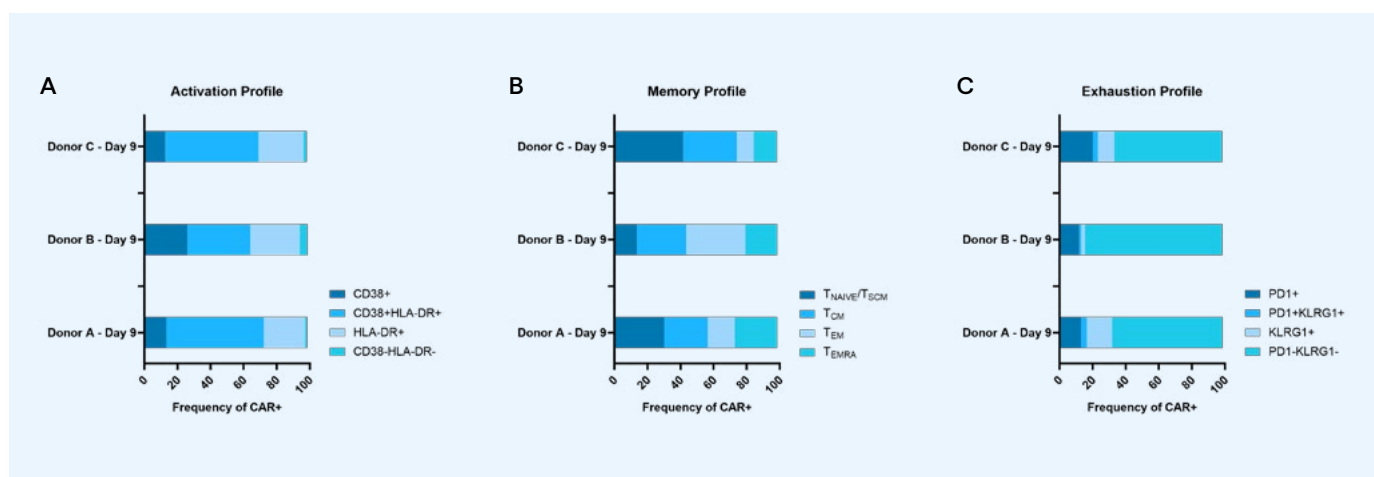


Figure 4: Cocoon® Platform manufactured CAR-T cell activation, memory, and exhaustion profiles:

A) Day-9 Cocoon® Platform manufactured CAR-T cells showed robust activation profile with upregulation of one or both activation markers (CD38 and HLA-DR) for all three donor products.

B) Memory phenotypes captured the various memory T-cell subsets present in the final Cocoon® Platform CAR-T product. A substantial proportion of stem cell memory and central memory T cells ($T_{NAIVE/SCM}$

and T_{CM} respectively) was observed in the day 9 product. $T_{NAIVE/SCM}$: CD45RA+CCR7+, T_{CM} : CD45RA-CCR7+, T_{EM} : CD45RA-CCR7-, T_{EMRA} : CD45RA+CCR7-.

C) Cocoon® CAR-T cell products demonstrated low expression of exhaustion markers (PD1 and KLRG1) with only a small subset of T cells co-expressing both the exhaustion markers (less than 5% of the CAR-T product) in all three donors.

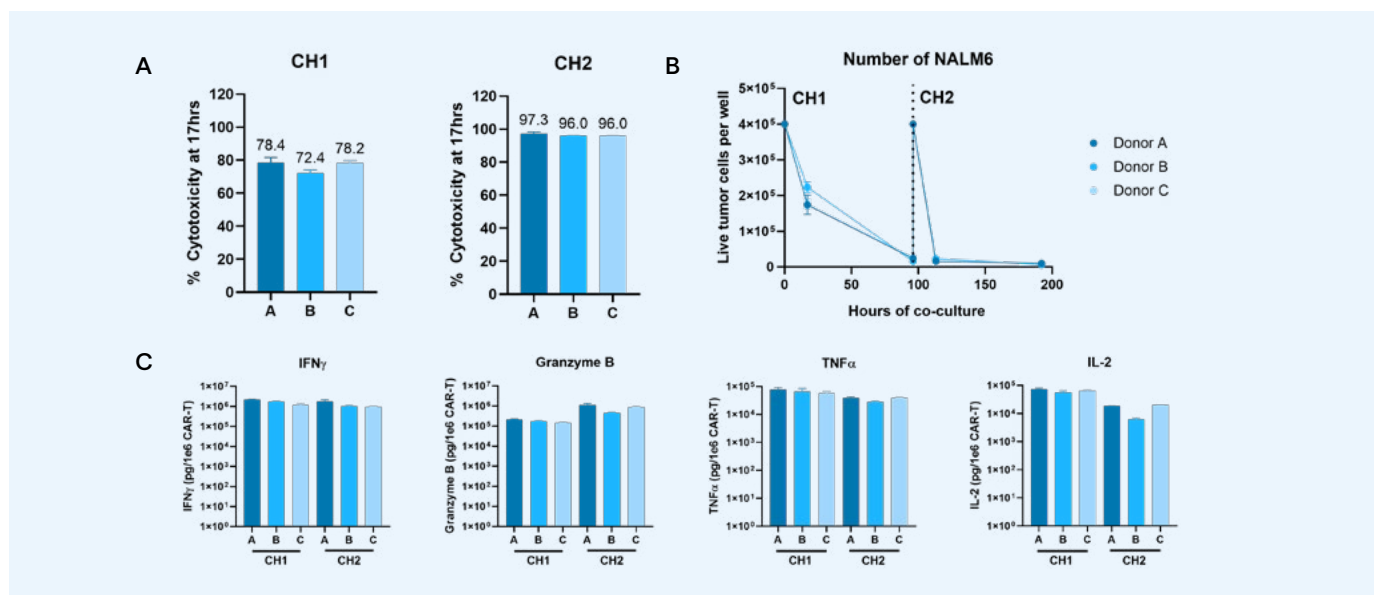


Figure 5: Assessment of CAR-T cell potency in co-culture with NALM6 tumor cells:

A) CAR-T cells manufactured on the Cocoon® Platform in serum-free media demonstrated potent CAR-mediated anti-tumor response during the initial challenge (CH1) with NALM6 tumor cells at 1:2 effector-to-target (E:T) ratio. Even more pronounced response was observed upon second challenge (CH2) at the same E:T ratio, highlighting a robust recall response and functional persistence of these CAR-T cell products.

B) Kinetics of tumor clearance over time is shown for each donor for the two sequential challenges (CH1 and CH2).

C) Cocoon® Platform manufactured CAR-T products showed robust cytokine production in response to tumor in both challenges. Within the first 17-hrs of co-culture, IL-2 production was observed (suggesting T cell activation) and notable levels of IFN γ , Granzyme B, and TNF α were detected, demonstrating induction of cytotoxic signaling.

Flexible Process Design

The Cocoon® Platform serum-free CAR-T application is also compatible with selected T cell input material through integrated selection in the Cocoon® Platform using CTS™ Detachable Dynabeads™ CD3/CD28 or CD4/CD8. Customizable process design can enable various process durations including rapid manufacturing of the CAR-T products. The process can be tailored to suit specific requirements such as culture media, activation reagent, cytokines, viral vector, and transduction enhancers.

Contributors

Samatha Bandapalle, Sree Pulugulla, Rahul Purohit, Jerison Jebaraj, Chengkang Zhang, Janet J. Sei, Tamara Laskowski

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