



Application note

Rapid Cocoon® CAR-T™: rapid manufacturing of CAR-T cells with integrated selection in the Cocoon® Platform

Automated T cell selection and CAR-T cell rapid manufacturing

Integrated magnetic separation capabilities enrich for a pure T cell input

To enable rapid manufacturing (RM) of CAR-T cells, an upstream T cell selection step is often preferred to ensure generation of a high-purity product. The Octane Cocoon® Platform is an automated solution that enables rapid manufacturing of CAR-T cells in a streamlined process that comprises T cell enrichment, activation, transduction, and final product harvest performed in a single-use, functionally-closed cassette (Figure 1). The use of CTS™ Detachable Dynabeads™ for T cell selection enhances control over the bead release / removal timepoints, thus providing flexibility and improved process efficiency.

In the Cocoon® Cassette, a magnetic interface captures bead-bound cells allowing selection of desired cell types. By integrating a CD3/CD28 positive selection strategy, we can enrich T cells (Figure 1) while simultaneously driving cell activation. Once the desired activation duration is achieved, beads are detached from the selected cells through a short incubation in release buffer.

In this application note, we describe a three-day process for rapid CAR-T cell manufacturing with integrated T cell selection in the Cocoon® Platform.



Automated Rapid Manufacturing (RM)

- Enables RM with integrated T cell selection on the Cocoon® Platform¹
- T cell selection is performed directly from apheresis material, without need for pre-processing
- RM Cocoon® CAR-T™ products show robust memory and activation profiles
- RM Cocoon® CAR-T™ show strong cytotoxicity, eliminating more than 50% of tumor cells (NALM6) within the first 17hr of co-culture

¹Modifications to the protocol may impact the total cell yield

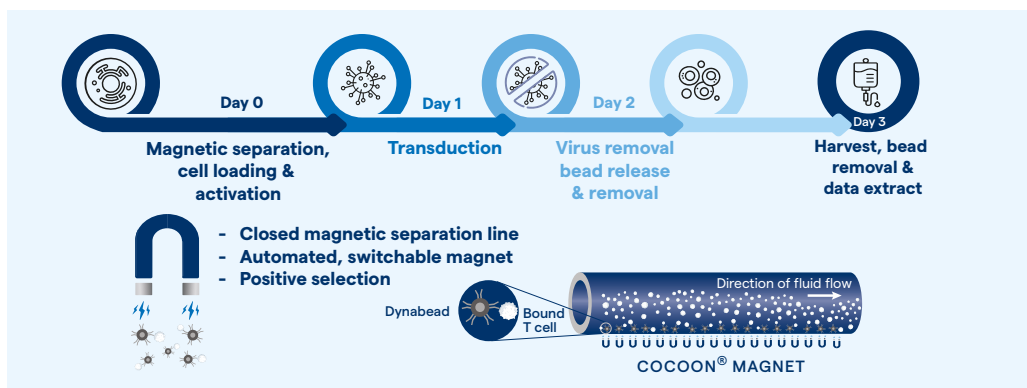


Figure 1: Rapid manufacturing in the Cocoon® Platform using CTS™ CD3/CD28 Detachable Dynabeads™.

The rapid Cocoon® CAR-T™ process integrates magnetic T cell selection to enable a fully automated solution. Following selection directly from apheresis material, bead bound T cells are seeded in the proliferation chamber to activate, and are subsequently transduced. Automated bead removal occurs at multiple timepoints prior to final product harvest at the end of the manufacture. Electronic batch records and data logs capture every step in the process and can be exported digitally.

Robust T cell selection and rapid manufacturing of CAR-T cells using the Cocoon® Platform

This three-day RM application enables automated magnetic separation, activation, transduction, media perfusion, bead removal, and final cell product harvest. Briefly, cells are incubated with CTS™ Detachable Dynabeads™ CD3/CD28 at a 1:1 bead-to-T cell ratio prior to positive selection. T cell selection occurs inside the Cocoon® Cassette. Additional process automation for integrated bead washing and binding in the Cocoon® Cassette reduces operator hands-on time and eliminates the need for additional equipment.

Rapid Cocoon® CAR-T™ process data for four cryopreserved healthy donor apheresis samples show the initial T cell

fraction (20-60%) and the subsequent enrichment achieved upon selection directly from the input apheresis material (Figures 2A-B), without need for pre-processing.

In an automated step, following T cell activation, release buffer is pre-warmed in the cassette then transferred to the cell layer for incubation and bead detachment. Cells are re-suspended and the detached beads are removed from the culture using the integrated Cocoon® Magnet. Automated bead-removal sequences are programmed into the Cocoon® protocol and executed at the harvest time point.

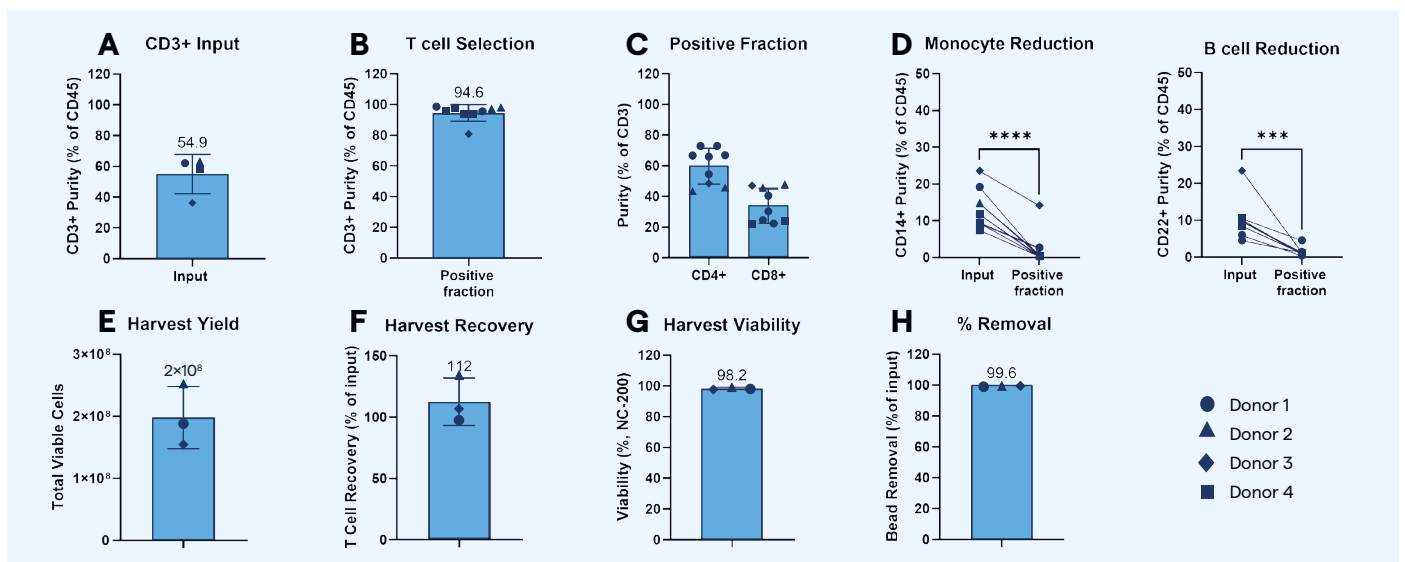


Figure 2: T cell selection and rapid manufacturing in the Cocoon® Platform

A) Healthy donor cryopreserved apheresis starting material was used in the Cocoon® RM process. The proportion of CD3+ T cells in the initial material ranged from 20-60% of CD45+ cells. On each bar graph, the symbol represents a distinct donor (n=4).

B) T cells were enriched using CTS™ Detachable Dynabeads™ CD3/CD28 to an average of 94.6±5.5% purity. An apheresis material containing low CD3+ T cell fraction (Donor 3) was also tested, and achieved robust enrichment. A total of nine independent selections on the Cocoon® Platform are shown. The mean with standard deviation is plotted.

C) The post-selection product fractions of CD4 and CD8 T cells are shown.

D) Cellular impurities are efficiently reduced through the selection process. Data show significant reduction of monocytes and B cells during the selection process. Statistics performed by paired t-test, ****p<0.0001, ***p<0.001.

E) Cells selected with CTS™ Detachable Dynabeads™ CD3/CD28 were seeded and activated in the cassette proliferation chamber after automated magnetic separation on day 0 (n=3). A total of 2.0 x 10⁸ viable selected cells were seeded on day 0, which resulted in a starting population of 1.60-1.92 x 10⁸ purified T cells. Cells were cultured in TheraPEAK™ X-VIVO™-15 medium supplemented with 5% human serum and 100 IU/mL IL-2 for 3 days. Recovery of input T cells on harvest was greater than 80% for n=3 donors.

F) Recovery of total T cells in the automated harvest on day 3 as a proportion of T cells seeded day 0 was 112±19.3%, showing evidence of early expansion during the three days of culture.

G) Cell counts were performed on the NC-200 with Via1 cassettes. High cell viability was maintained on the RM final product.

H) The Cocoon® Platform enables robust removal of CTS™ Detachable Dynabeads™. On average, 99.6% of input beads were removed by day 3 of the manufacturing process.

RM Cocoon® CAR-T™ Cell Phenotype

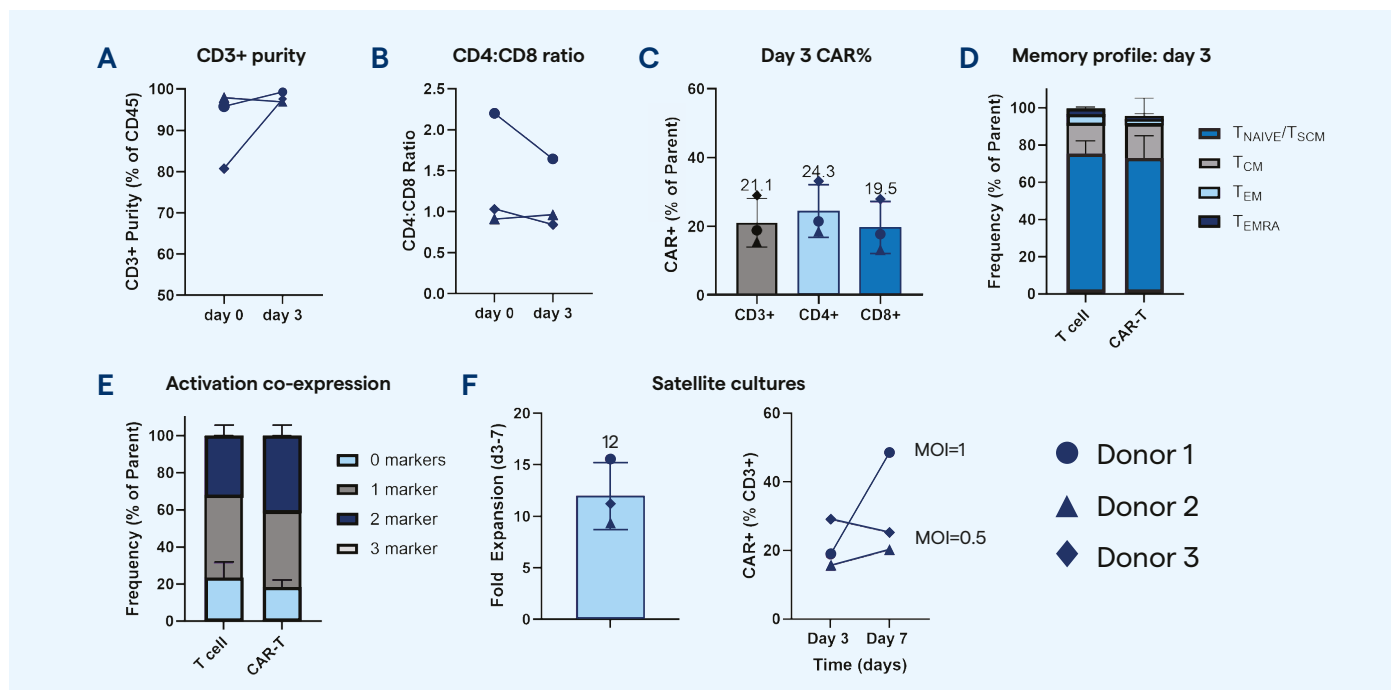


Figure 3: Cocoon® CAR-T™ cell phenotypic characterization

A) Greater than 95% CD3+ T cell purity was observed for three healthy donors on day 3 of the RM protocol.

B) Proportion of CD4+ and CD8+ T cells in the harvest product was generally maintained, albeit likely influenced by donor-intrinsic characteristics, as donor 1 exhibited greater expansion of CD8+ T cells by end of manufacture.

C) Following activation with CTS™ Detachable Dynabeads™ CD3/CD28, CAR-T cells were transduced at an MOI of 0.5 or 1 with a CD19-CAR lentivirus vector (manufactured at Lonza). CAR expression was detectable on day 3 in all donors. Transduction of both the CD4 and CD8 T cell subsets was observed.

D) Memory phenotype analysis by flow cytometry elucidated the different memory T cell subsets present in the final product. The average and standard deviation of three donors are shown. Large proportions of stem central memory and central memory T cells were observed in the Cocoon® CAR-T™ products. $T_{NAIVE/SCM}$ = CD45RA+CCR7+, T_{CM} = CD45RA-CCR7+, T_{EM} = CD45RA-CCR7-, and T_{EMRA} = CD45RA+CCR7-.

E) Flow cytometry analysis of Cocoon® T cell and CAR-T™ cell products showed upregulation of 1 or more activation markers (CD69, CD38 and HLA-DR). The average and standard deviation of three donors are shown.

F) The day 3 product was seeded into small-scale cultures to expand until day 7 and to monitor CAR-T cell proliferation. CAR expression in donors transduced at an MOI of 0.5 was overall stable from days 3-7. CAR expression in Donor 1, which was transduced at an MOI of 1, increased from day 3 to day 7.

The Cocoon® Platform enables process and protocol flexibility

The magnetic separation cassette in the Cocoon® Platform automates enrichment, activation, and transduction of T cells. Customizable process design can enable various process durations and allows users to modify cell seeding numbers and activation parameters. Applications can be tailored to suit specific requirements regarding culture media, cytokine, and activation reagent. In this application, we demonstrate efficient CAR-T cell transduction at low MOI (Figure 3 C,D) using RUO Lonza-manufactured lentivirus vector delivering a CD19-CAR. Transduction can be optimized in the Cocoon® Platform by modifying parameters such as vector input concentration, transduction media, and addition of enhancers.

Using the Cocoon® Platform to shorten manufacturing workflows to a rapid process reproducibly yields CAR-T cell products that maintain robust memory characteristics and activation profile (Figure 3 E,F). Moreover, Rapid Cocoon® CAR-T™ products exhibit strong cytotoxicity, expansion, and release of proinflammatory cytokines upon challenge with tumor cells (Figure 4).

Strong product stability and CAR-T cell-mediated killing by Cocoon® CAR-T™ cell products

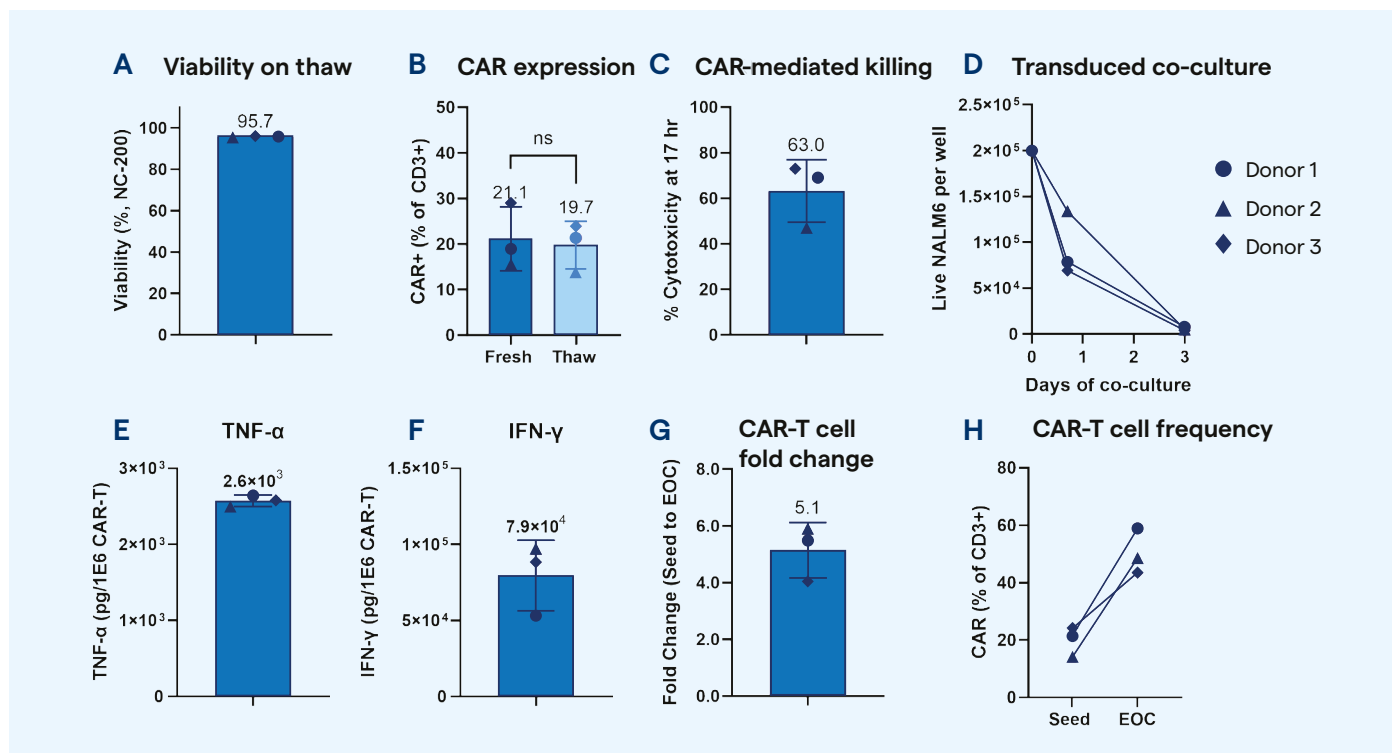


Figure 4: Rapid manufactured Cocoon® CAR-T™ cell functional characterization

A) Cryopreserved Cocoon® CAR-T™ cell products were thawed and analyzed to assess product stability and functional performance. High viability at greater than 95% was maintained post thaw.

B) Flow cytometry analysis of Cocoon® products showed stable CAR expression post thaw.

C) Potency assessments in co-culture of Cocoon® CAR-T™ products with NALM6 tumor cells at 1:2 effector-to-target cell ratio. CAR-T cells showed potent response against NALM-6 tumor cells achieving greater than 50% cytotoxicity within the first 17 hours.

D) Kinetics of Cocoon® CAR-T™ cell-driven anti-tumor response showed fast initial killing within the first 17 hours and total tumor clearance by the end of assay (3 days).

E,F) Cocoon® CAR-T™ products exhibited strong TNFα and IFNγ production profiles in response to tumor challenge, supporting the robust cytotoxic response observed upon engagement with NALM6 tumor targets.

G) CAR-T cell expansion in culture was driven by activation upon tumor engagement as no exogenous cytokines were added. T-cell-alone control cultures showed no expansion (data not shown).

H) During co-culture with NALM6 tumor cells, CAR-T cells increased in frequency as they proliferated and persisted to drive robust anti-tumor response until the end of culture (EOC) on day 3.

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