



Application note

End-to-end production of T cells on the Cocoon® Platform, using buoyancy-activated Cell Sorting™ for T cell isolation from leukopaks

The Cocoon® Platform enables isolation, modification, and expansion of T cells in a functionally-closed automated system. By incorporating Akadeum Life Sciences® Buoyancy-Activated Cell Sorting (BACS™) technology, the Cocoon® Platform now enables negative T cell selection. The process enriches CD3+ T cells without direct biochemical interaction with exogenous materials. This preserves the T cells' innate, unstimulated state, and allows users to customize the activation reagents and strategies.

Automated T cell enrichment on the Cocoon® Platform features the use of a GMP-grade antibody mixture (CD14, CD16, CD19, CD20, CD36, CD56, CD123, CD235ab) and buoyant microbubbles.

Application summary

Automated cell separation

- The Cocoon® Platform enables isolation of T cells directly from cryopreserved leukopaks
- No additional upstream equipment required
- Reduced operator interactions
- Negative selection enables flexibility of activator
- Recover ~64% of CD3+ cells with ~92% purity¹
- Expand to $\sim 2.6 \times 10^9$ T cells in 9 days¹

¹ Cell source and modifications to the protocol may impact total cell yields

The unwanted cells bind to the microbubbles and float to the surface of the liquid, enabling the removal of non-T cells from the subnatant. Following the BACS™ process on the Cocoon® Platform, excess cells are removed from the system to provide control over the initial number of T cells that are seeded. This remaining T cell population is then activated and expanded (**Figure 1**).

Here, we describe a 9-day automated T cell isolation and manufacturing process on the Cocoon® Platform using the GMP-grade Microbubble Leukopak Human T Cell Isolation Kit from Akadeum® (13210-221GMP). Quarter-sized cryopreserved leukopaks (CryoStor® CS10) were selected to demonstrate functionality.

In this process, following leukopak thawing, the operator loads antibodies to the leukopak and attaches this to the Cocoon® Platform. The operator then attaches a separate bag of microbubbles. The Cocoon® Platform automates the enrichment of T cells in 40 minutes by controlling mixing, incubations, and fluid transfers (**Figure 2**). Following the BACS™ process, the operator removes a sample for counting. 1×10^8 T cells are automatically transferred to an expansion chamber and cultured using media supplemented with 5% human serum and IL-2. T cells were stimulated using a CD3/CD28 T cell activator. Two operators executed the presented workflow (mock transduction) using leukopak materials obtained from six donors (n=6).

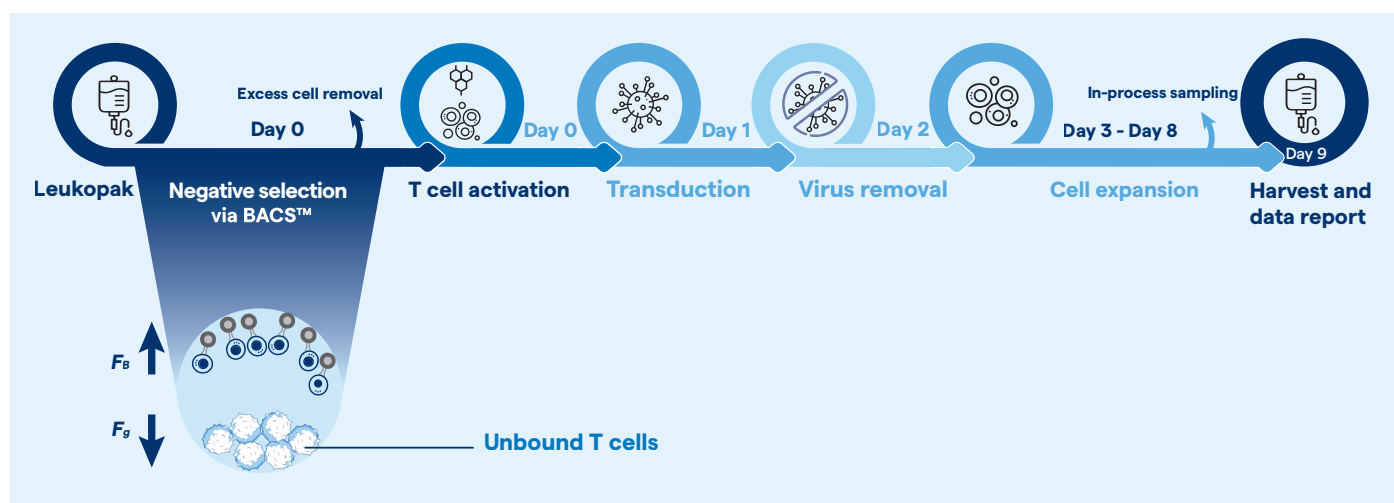


Figure 1: The Cocoon® Platform executes an automated 9-day protocol to inoculate, isolate, activate, transduce, and expand T cells. This process features the use of BACS™ technology to isolate T cells. Unwanted cells are bound to microbubbles enabling the use of buoyant force (F_b) against the force of gravity (F_g). The Cocoon® Platform continues the process by automating media exchanges, dissolved oxygen and pH monitoring, biofeedback, in-process sampling, and final cell product harvest.

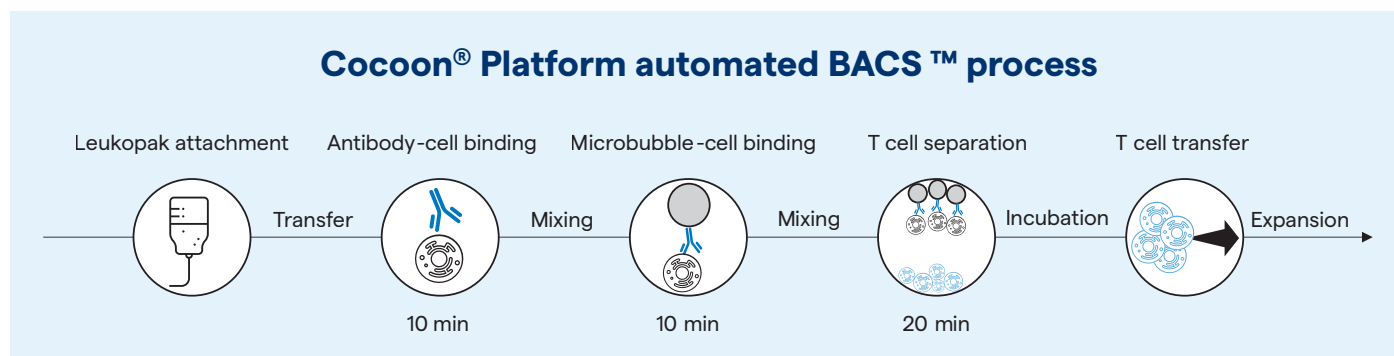


Figure 2: Automated Buoyancy-Activated Cell Sorting (BACS™) on the Cocoon® Platform. Following BACS™ separation, T cells are expanded.

Performance data

Donor materials ranging from 50% to 70% CD3+ cells were purified to 88% to 97% (**Figure 3A**). The automated sorting process yielded 50% to 86% of T cells from the original material and reduced non-T cell populations, CD14+, CD19+ and CD56+, to 1.7%, 0.5% and 5.5% respectively (**Figure 3A and B**). CD3+ cell viability exceeded 97% in all runs after sorting (**Figure 3C**).

After enrichment, T cells were expanded from 1×10^8 to $\sim 2.6 \times 10^9$ viable T cells within 9 days (**Figure 4A**). Throughout expansion, T cell viability and CD3+ purity exceeded 90%. The CD3+CD4+ and CD3+CD8+ T cell population fractions were within the expected parameters (**Figure 4B and 4C**).

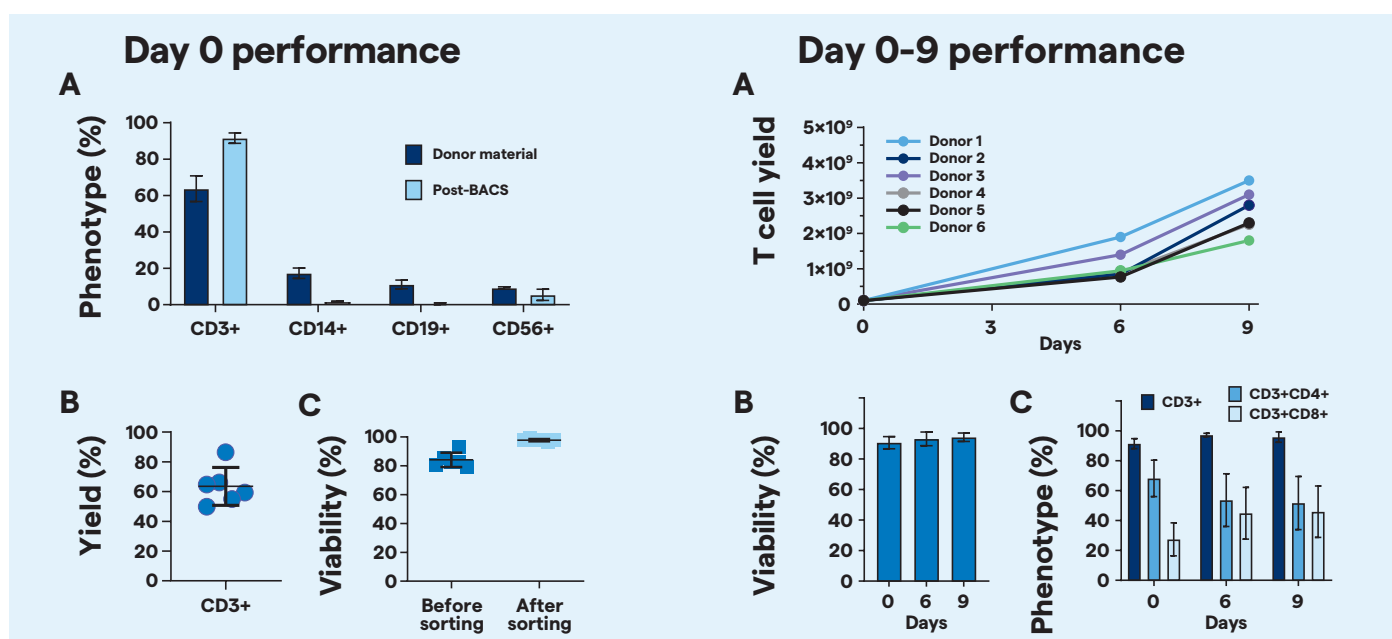


Figure 3: Non-T cell reduction and viable T cell yield post-BACS™ on the Cocoon® Platform. (A) Non-T cell population fractions (n=6) before and after BACS™ acquired using flow cytometry and automated cell counters. (B) Day 0 viable T cell yield after BACS™ (n=6). (C) Cell viability before and after BACS™ (n=6).

Figure 4: Automated 9-day T cell expansion on the Cocoon® Platform yields on average 2.6×10^9 T cells. (A) Viable cell yield. (B) T cell viability. (C) Purity and CD3+CD4+ and CD3+CD8+ populations within viable CD3+ cells. Data acquired via flow cytometry and automated cell counters (n=6).

In summary, the Cocoon® Platform automates the T cell selection process from donor leukapheresis materials using BACS™ technology by Akadeum Life Sciences®. By enabling processing directly from a leukopak, operator time is reduced and the requirement for additional upstream processing equipment is removed.

Following automated, flotation-based cell separation, 1×10^8 T cells were activated and expanded to $\sim 2.6 \times 10^9$ T cells in a fully automated process on the Cocoon® Platform. The process features automated T cell sorting, fluid mixing and fluid transfers, which provide the user with an end-to-end experience from leukopak to harvest, requiring minimal user interaction.

Contributors

Salvador Flores-Torres, Michele Vicentini-Hogan, Peter Szaraz, Matteo Da Ros, Carlene Petes, and Nuala Trainor.

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