

# Automated T cell enrichment on the Cocoon® Platform using magnetic separation

Magnetic Separation Cassette yields reproducible automated CD3+ cell selection

## Integrated magnetic separation capabilities for enriching a pure T cell population

Magnetic separation is a common, optional upstream process step for T cell therapy manufacturing, used to enrich a CD3+ cell population for homogeneous starting material while non-CD3 expressing cells are removed. Lonza's Cocoon® Platform, including the functionally-closed Magnetic Separation Cassette, is a customizable, automated solution for T cell enrichment, activation, transduction, expansion, medium exchanges, bead removal, culture washing, and final product harvest (Figure 1).

The cassette is a single-use disposable consumable with a fluidic selection tube that aligns with a switchable magnet located inside the Cocoon® Instrument. The magnetic interface draws bead-bound cells to the tube wall, allowing selection of desired cell types and the removal of unwanted phenotypes (Figure 2). The process can be customized for positive or negative selection.

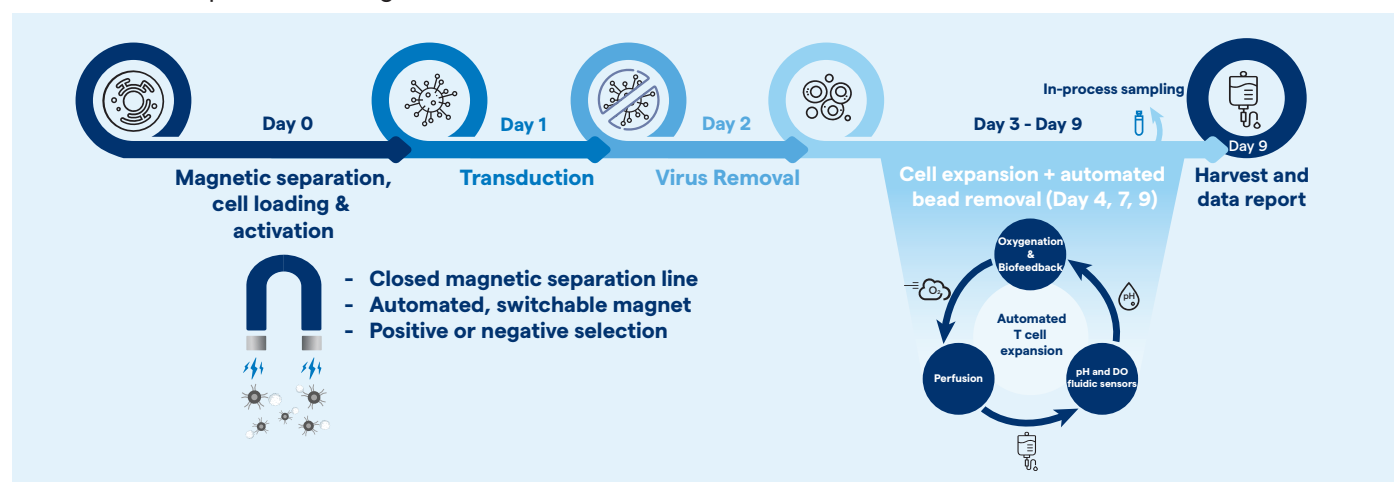
## Application summary

### Automated magnetic separation

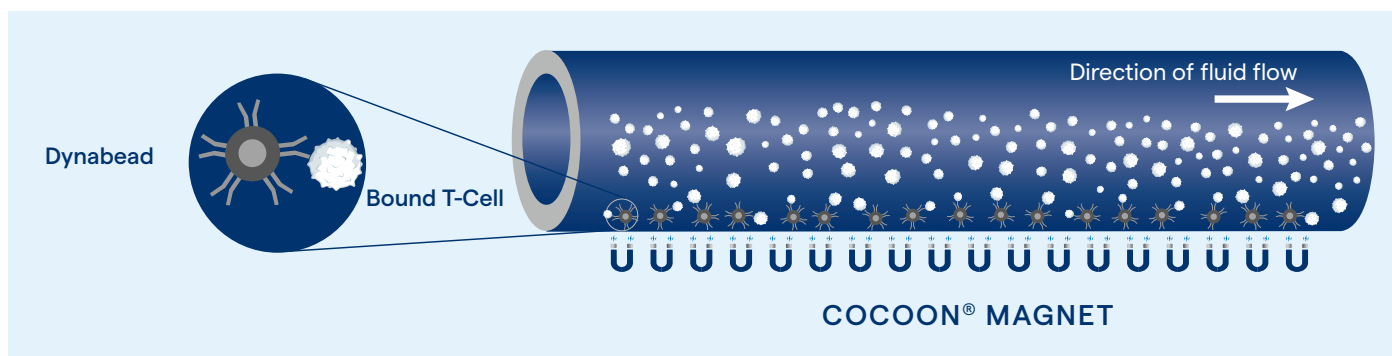
- Off-the-shelf, automated T cell enrichment workflow for magnetic separation, activation, and expansion on the Cocoon® Platform
- Reliably enrich >80% pure CD3+ T cells
- Consistent viabilities >90% in the positively selected CD3+ fraction
- Expand to  $2.3 \times 10^9$  T cells in 9 days<sup>1</sup>, maintaining >85% cell viability
- Bead removal efficiency >99%

<sup>1</sup> Modifications to the protocol may impact total cell yields

The following application note describes a standard automated positive selection 9-day process for T cell therapy manufacturing using CTS™ Dynabeads™ CD3/CD28 (Thermo Fisher Scientific) for upstream T cell enrichment on the Cocoon® Platform.



**Figure 1: Process overview of the Octane standard T cell magnetic separation protocol using CTS™ CD3/CD28 Dynabeads™ on the Cocoon® Platform.** The Cocoon® Platform runs customizable processes in a functionally-closed, single-use disposable cassette for automated magnetic separation, cell loading, activation, transduction, Dynabeads™ removal, cell expansion, cell washing and final product harvest. During cell expansion, the Cocoon® Platform has programmable biofeedback features to maintain a balanced pH. The Cocoon® Platform utilizes fluidic dissolved oxygen (DO) and pH sensors to monitor the cell culture environment. At the end of the process, electronic batch records and data logs can be exported digitally.



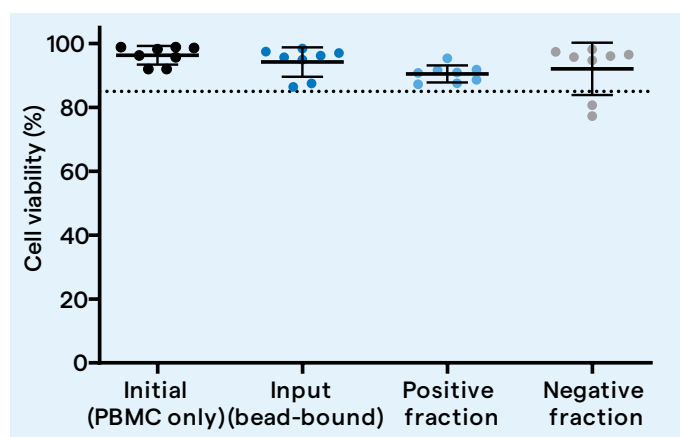
**Figure 2: Magnetic separation on the Cocoon® Platform.** PBMC incubated with Dynabeads™ in cell suspension flow through the cassette magnetic separation line. Automated Cocoon® Platform magnets attract bead-bound cells to the tube wall and non-bead bound cells are flushed to waste. Upon turning off the magnet, desired bead-bound cells are seeded in the cassette for further processing (image is not to scale).

## Performance Data

### Reliably select target cells with high viability and purity on the Cocoon® Platform

Automated magnetic separation was performed on the Cocoon® Platform using cryopreserved peripheral blood mononuclear cells (PBMC) starting material from four independent donors. Cells were incubated with CTS™ Dynabeads™ CD3/CD28 at a 1:1 bead:cell ratio for T cell enrichment using positive selection. CD3+ cell selection occurred inside the closed magnetic separation fluidic line on the cassette using the automated, switchable magnet in the Cocoon® Instrument.

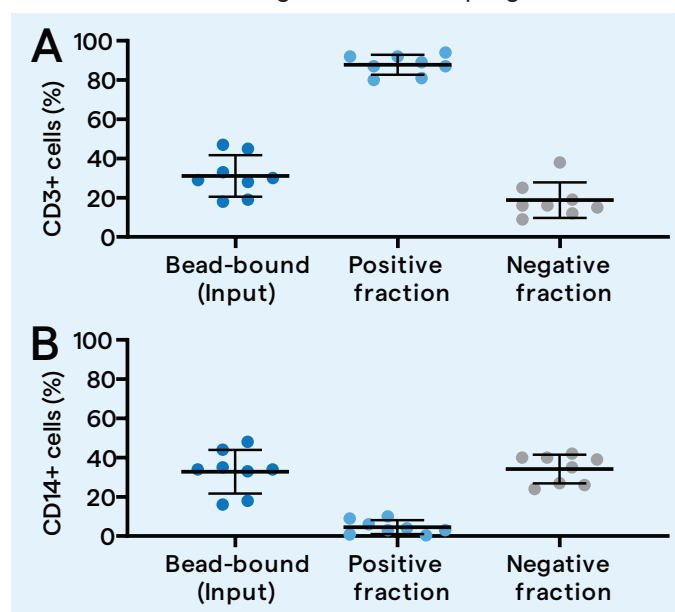
Throughout the magnetic separation process, cell viabilities were maintained on average above 90% (Figure 3). Specifically, the positive selected fraction contained on average 91% viable cells post-selection.



**Figure 3: Cell viabilities before and after magnetic separation on the Cocoon® Platform.** Cell viabilities were assessed before and after magnetic separation. Each dot represents one data point (n=8). Bold lines and error bars represent the mean and standard deviation, respectively. Dotted line represents 85% viability.

Automated magnetic separation on the Cocoon® Platform reproducibly achieved average purity levels of 88% selected CD3+ cells in the positive fraction (Figure 4A). Low CD14+ residual cells (<5%) remained in the positive fraction (Figure 4B).

Positive fractions of bead-bound T cells were subsequently activated, transduced, expanded, washed, and harvested with in-process bead removal on the Cocoon® Platform using an automated program.

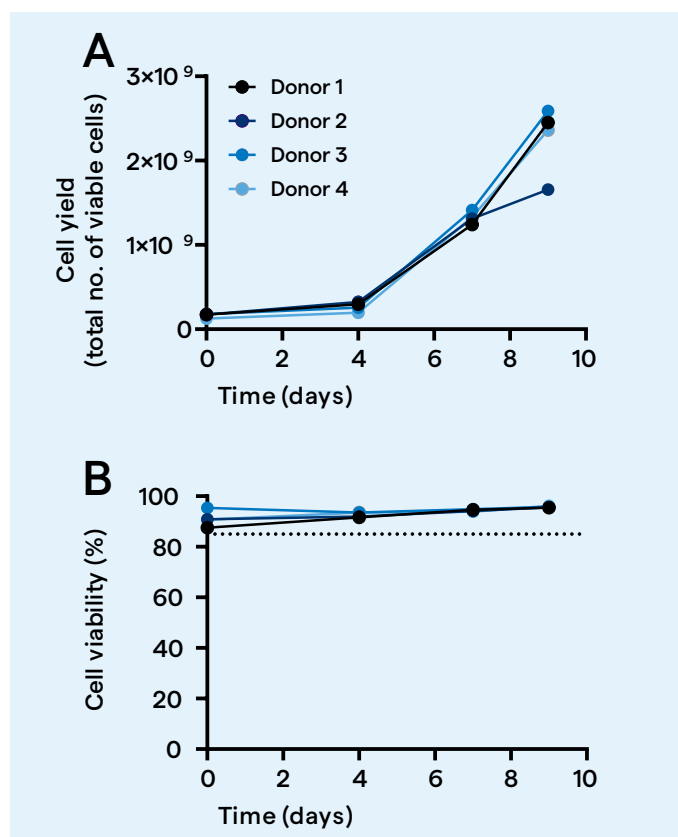


**Figure 4: CD3+ T cell purity and CD14+ monocyte residuals following magnetic separation on the Cocoon® Platform.** Using flow cytometry, frequencies of CD3+ cells (A) and CD14+ cells (B) were quantified in the bead-bound starting PBMC material (input), positive (CD3+) and negative (CD3-) fractions to assess the cell phenotypes. Each dot represents one data point (n=8). Bold lines and error bars represent the mean and standard deviation, respectively.

## The automated Cocoon® Platform delivers consistent T cell expansion post magnetic separation and includes bead removal functionality

The Cocoon® Platform achieves robust expansion of purified T cells following in-line automated enrichment and bead removal steps. The automated 9-day protocol includes magnetic separation, activation, transduction, expansion, media perfusion, Dynabeads™ removal, final cell product wash and harvest (Figure 1).

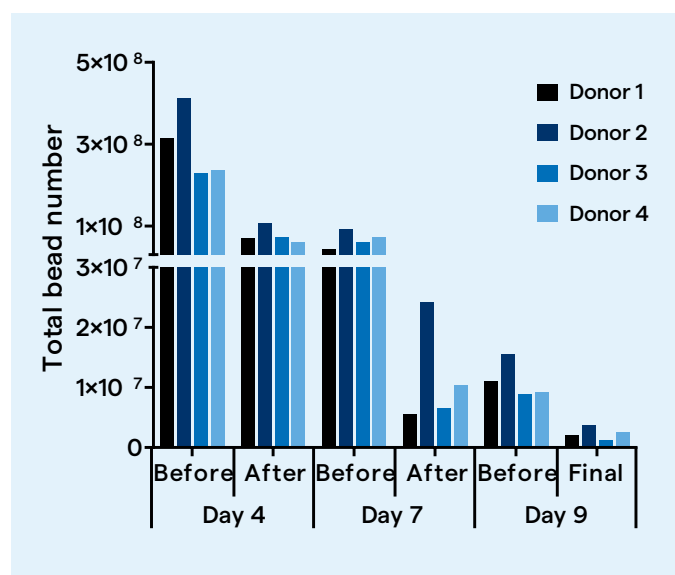
Starting with a range of  $1.3 \times 10^8$  to  $1.8 \times 10^8$  enriched T cells seeded in the cassette on day 0, the culture expanded to  $1.7 \times 10^9$  to  $2.6 \times 10^9$  T cells in the final cell product on day 9 (Figure 5A). On average,  $2.3 \times 10^9$  cells expanded in the cassette using Lonza's TheraPEAK™ X-VIVO™-15 cell culture medium supplemented with 5% human serum and 100 IU/mL IL-2. Cell viabilities were maintained above 85% throughout the 9-day protocol, as assessed at cell seeding, cell sampling on days 4 and 7 and in the final cell product harvested on day 9 (Figure 5B).



**Figure 5: Automated 9-day T cell expansion on the Cocoon® Platform yields on average  $2.3 \times 10^9$  T cells.** CD3+ cells bound to CTS™ Dynabeads™ CD3/CD28 were seeded and activated in the cassette after automated magnetic separation on day 0 (n=4). In-process Dynabeads™ removal and sampling for cell counts were performed on days 4, 7, and 9 after bead removal. Cells were expanded to day 9 to assess cell yield (A) and viability (B) (n=4). Dotted line represents 85% viability.

Removal of CTS™ Dynabeads™ CD3/CD28 in a T cell therapy manufacturing process is essential to avoid over-activation of T cells, which could negatively impact subsequent T cell viability and expansion. Dynabeads™ separate naturally from T cells over time as the CD3 protein on the cell surface is recycled. Importantly, Dynabeads™ must be removed from the final product to minimize residuals prior to product formulation. To reduce the number of Dynabeads™ in the final product, three automated bead removal sequences are programmed in the Cocoon® protocol on days 4 and 7 as well as during harvest on day 9.

In the Octane standard magnetic separation protocol, cell samples are collected from the cassette at the time of each automated bead removal sequence. To assess efficiency of Dynabeads™ removal throughout the process, samples were collected before and after bead removal on days 4, 7 and 9 to be analyzed for total Dynabeads™ counts. Bead removal on the Cocoon® Platform reduced the total Dynabeads™ residuals from an average of  $3.0 \times 10^8$  beads to an average of  $2.4 \times 10^6$  beads in the final product (Figure 6). The average bead removal translates to greater than a 99% reduction of beads from days 4 to 9.



**Figure 6: Automated bead removal on the Cocoon Platform minimizes residual CTS™ Dynabeads™ through the 9-day protocol.** Dynabeads™ counts were assessed in cell samples collected from the resuspended culture before and after automated bead removal steps on days 4, 7 and 9. Counts were quantified using a hemocytometer (n=4).

# Summary

The Cocoon® Platform with the Magnetic Separation Cassette efficiently enriches, activates, and expands T cells from PBMC starting material. Automated steps are performed in one functionally-closed, single-use disposable cassette throughout the 9-day standard magnetic separation Cocoon® protocol. The Cocoon® Platform achieves on average 88% CD3+ purity and 91% CD3+ cell viability using CTS™ Dynabeads™ CD3/CD28. Moreover, the Cocoon® Cassette enables robust T cell activation and expansion achieving on average  $2.3 \times 10^9$  T cells, while maintaining cell viabilities greater than 85%. Customizable, automated bead removal steps eliminate over 99% of Dynabeads™ in the final cell product following the 9-day protocol.

## Tips for successful automated magnetic separation on the Cocoon® Platform

The automated Cocoon® protocol for magnetic separation was optimized using CTS™ Dynabeads™ CD3/CD28 for CD3+ cell selection, viability, purity, and expansion, while minimizing residual Dynabeads™. Cocoon® specific process details are listed in the table below:

**Table 1. Process details for implementation of the automated magnetic separation 9-day protocol on the Cocoon® Platform.**

| Cocoon® process              |                                                                                                                                | Benefits                                                                                                                                                                                                                                                                                                                                                               |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Bead:cell ratio              | Bead-binding ratio of 1:1 beads per T cell.                                                                                    | <b>The Octane Magnetic Separation Cocoon® protocol enables:</b> <ul style="list-style-type: none"><li>• An easy to follow consistent, automated workflow</li><li>• Low Dynabeads™ residuals, while maintaining consistent CD3+ cell selection and purity</li><li>• High cell recovery and viability throughout the process</li><li>• Robust T cell expansion</li></ul> |
| Bead binding                 | Perform bead binding of PBMC containing $400\text{--}600 \times 10^6$ T cells in a 70mL semi-permeable bag.                    |                                                                                                                                                                                                                                                                                                                                                                        |
| Cell loading                 | Cells incubated with Dynabeads™ are loaded directly from the bag into the cassette fluidics for automated cell selection.      |                                                                                                                                                                                                                                                                                                                                                                        |
| Positive fraction collection | Positive fraction T cells are collected in a semi-permeable bag in expansion media attached directly to the cassette fluidics. |                                                                                                                                                                                                                                                                                                                                                                        |
| Bead removal                 | Multiple automated bead removal sequences throughout the process.                                                              |                                                                                                                                                                                                                                                                                                                                                                        |

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