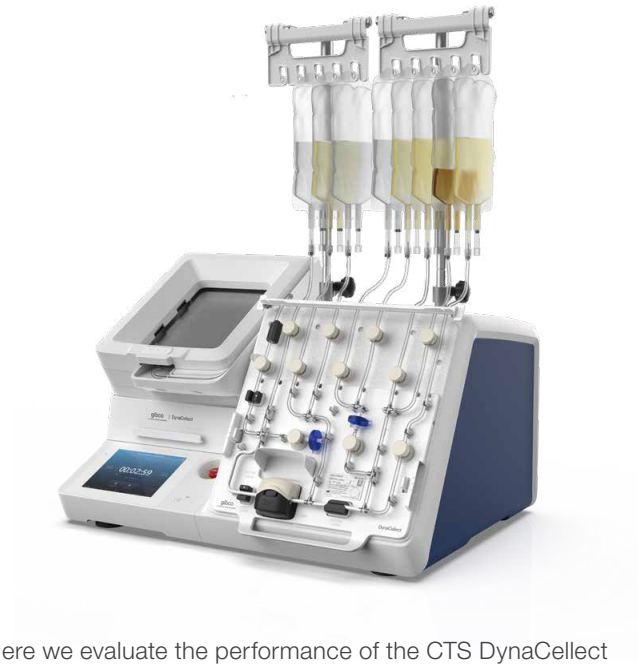
Cell therapy solutions

Automated and closed CTS DynaCellect Magnetic Separation System delivers target T cell isolation and magnetic bead removal at high efficiency



Introduction

Successful commercial manufacturing of T cell therapies is essential to assuring the timely delivery of efficacious products to patients. Adequate cell therapy workflows have been developed to support clinical trials and commercialized T cell therapies. However, the industry faces several key barriers that limit the capability to deliver the required volume of high-quality product for autologous therapies and developing allogeneic applications [1]. Challenges may include assurance of consistent product quality and efficacy during scaling, risks in manual operating, lack of workflow flexibility and speed, and development of standardized processes that are regulatory-compliant.

To address key industry challenges, the Gibco™ CTS™ DynaCellect™ Magnetic Separation System, along with the Gibco™ CTS™ DynaCellect™ Cell Isolation Kit (Cat. No. A52300) and Gibco™ CTS™ DynaCellect™ Bead Removal Kit (Cat. No. A52301), were developed (Table 1). This cell processing platform provides an automated, closed, flexible, and scalable platform for T cell isolation and activation using magnetic beads.

Here we evaluate the performance of the CTS DynaCellect system when used with Gibco™ CTS™ Dynabeads™ CD3/CD28, with regards to T cell isolation yield, isolation efficiency, cell viability, target cell recovery, and potential time savings. Additionally, we discuss how the system's capabilities and performance can support industry challenges.

Table 1. Key manufacturing challenges and solutions.

Challenge	Solution
Manual, labor-intensive, and open processes	Closed and automated system processes with single-use kits
Product quality and regulatory compliance	Processes that achieve high yields of viable target T cells, effective activation, strong expansion, and high cell recovery, with regulatory support documentation
Rigid, unscalable processes	Flexible, modular, and scalable system processes
Slow and inefficient manufacturing processes	Time savings with faster and automated processes

Materials and methods

Reagents, medium, and cells

The dilution and wash buffer was Gibco™ CTS™ Dulbecco's Phosphate Buffered Saline (DPBS, Cat. No. A1285601) with 0.1% human serum albumin (HSA).

The complete medium consisted of Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM, no phenol red (Cat. No. A3705003), supplemented with the provided 2.6% Gibco™ CTS™ OpTmizer™ Expansion Supplement, 2.5% Gibco™ CTS™ Immune Cell Serum Replacement (ICSR, Cat. No. A2596102), 2 mM Gibco™ L-Glutamine (Cat. No. 2503008), and 12.5 µg/mL Gibco™ Gentamicin Reagent Solution (Cat. No. 15710049).

Cells were processed from leukopaks (HemaCare) containing peripheral blood mononuclear cells (PBMCs) from four healthy donors.

Kit preparation

The single-use CTS DynaCellect Cell Isolation Kit (Cat. No. A52300) and CTS DynaCellect Bead Removal Kit (Cat. No. A52301) were prepared by welding prefilled reagent bags to the appropriate kit tubing lines. The reagent bags were then hung on the appropriate hanger. The isolation or bead removal bag was installed into the rocker nest, and the tubes were installed into their respective pinch valves, bubble sensors, pressure sensors, and pump locations.

Isolation

Frozen leukopaks were thawed and diluted 1:1 with dilution buffer and counted on a MultiSizer 3 Particle Counter (Beckman Coulter). A cell input of 40 mL was calculated to obtain 10 x 10^6 $CD3^+$ cells/mL, based on the CD3 percentage provided in the supplier Certificate of Analysis. The CTS Dynabeads CD3/CD28 beads (Cat. No. 40203D) were used at a bead-to-cell ratio of 3:1 in the DynaCellect system with the isolation workflow summarized in Figure 1. The T cell isolation protocol was written using the embedded graphical user interface (eGUI) as outlined in the user manual. Processing time for isolation was approximately 1 hour. After isolation, the cells were resuspended in complete medium. The isolation efficiency, cell viability, purity, and activation were assessed as indicated in the performance criteria section.

Activation and expansion

Isolated cells from two donors were separately expanded and activated for 7 days in a G-Rex™ 100 system (Wilson Wolf) according to the manufacturer's protocol, with a 1,000 mL total final volume of complete medium supplemented with 100 U/mL of Gibco™ Recombinant Human IL-2 (Cat. No. CTP0021). The cells were incubated at 37°C with 5% CO_2. An additional 100 U/mL of IL-2 was added every 2 to 3 days.

Bead removal and cell recovery

Bead removal was performed on day 7 using the DynaCellect system to recover the expanded cells. The concentration and volume of the expanded cell suspension were measured and used to calculate the cell recovery relative to the input. Cells were counted on a MultiSizer 3 Particle Counter (Beckman Coulter). The suspension was transferred to a 1.0 L transfer bag for the bead removal process. The recovery protocol was written with the eGUI and conducted as described in the cell recovery workflow shown in Figure 2. The percent recovery of T cells was assessed as indicated in the performance criteria section. Bead removal processing times for both 1 L and 10 L volumes were also evaluated.

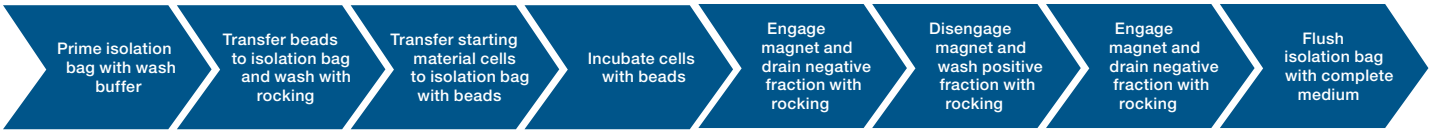


Figure 1. Summary of isolation workflow.

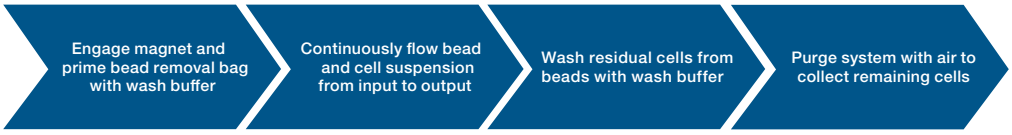


Figure 2. Summary of recovery workflow.

Performance criteria

- **Cell viability**—The CD45$^+$ cell viability of the starting material and the day 1 target cells were assessed by flow cytometry using an Invitrogen™ cell viability stain.
- **Phenotyping**—Phenotyping was performed by flow cytometry on the starting material and the isolated positive fraction on day 1 using Invitrogen™ monoclonal antibodies to determine the populations of T cells (CD2, CD4, and CD8), monocytes (CD14), B cells (CD19), and natural killer cells (CD56).
- **Activation**—Early CD69 and late CD25 activation markers were used to measure the activation level of the starting material on day 0, and the activation level of the isolated material on day 1 and day 7, using flow cytometry with Invitrogen™ CD69 and CD25 monoclonal antibodies.
- **Isolation efficiency**—The cell counts and volumes of the starting material and the negative fractions were measured. The average percentage isolation efficiency of CD3$^+$ CD28$^+$ cells was calculated based on the following equation:

$$\text{Isolation efficiency (\%)} = 1 - \left(\frac{\text{\% avg. CD3}^+\text{ CD28}^+\text{ in negative fraction x (avg. count x volume)}}{\text{\% avg. CD3}^+\text{ CD28}^+\text{ in input x (avg. count x volume)}}\right) \text{x } 100\%$$

- **Recovery**—The percentage of recovered cells was calculated using the following equation, where the total output and input cells are calculated by multiplying the cell concentration in cells/mL by the culture volume in mL.

$$\text{Recovery (\%)} = \frac{\text{Total output cells}}{\text{Total input cells}} \text{ x } 100$$

Results

In the isolation workflow, PBMCs with a 52% average T cell population were enriched in the CTS DynaCellect system with CTS Dynabeads CD3/CD28 to yield a 97% average target T cell population (Figure 3). Assessment of isolation efficiency showed an 86% combined average, with the four individual results ranging from 79% to 94% (Figure 4). In the isolation workflow, an 87% combined average cell viability was maintained from the starting to the isolated target cell material (Figure 5). As shown in Figure 6, evaluation of activation markers demonstrated the expected target CD69 and CD25 cell kinetics. The earlier CD69 marker was expressed at 90% on day 1 and subsequently downregulated to 7% by day 7. The later CD25 activation marker showed 89% expression on day 1 and continued upregulation and expression at 99% by day 7. After isolation in the system, a high average 88-fold T cell expansion was achieved with donor cells at the 1 L scale (data not shown). After bead removal, a 91% combined average target cell recovery was demonstrated with individual donor recovery results ranging from 87% to 95% (Figure 7). Lastly, total time savings of 83 min for cell recovery was realized with scale-up of bead removal to 10 L. The process rate was reduced from 29 min/L at the 1 L scale to approximately 21 min/L at the 10 L scale (Figure 8).

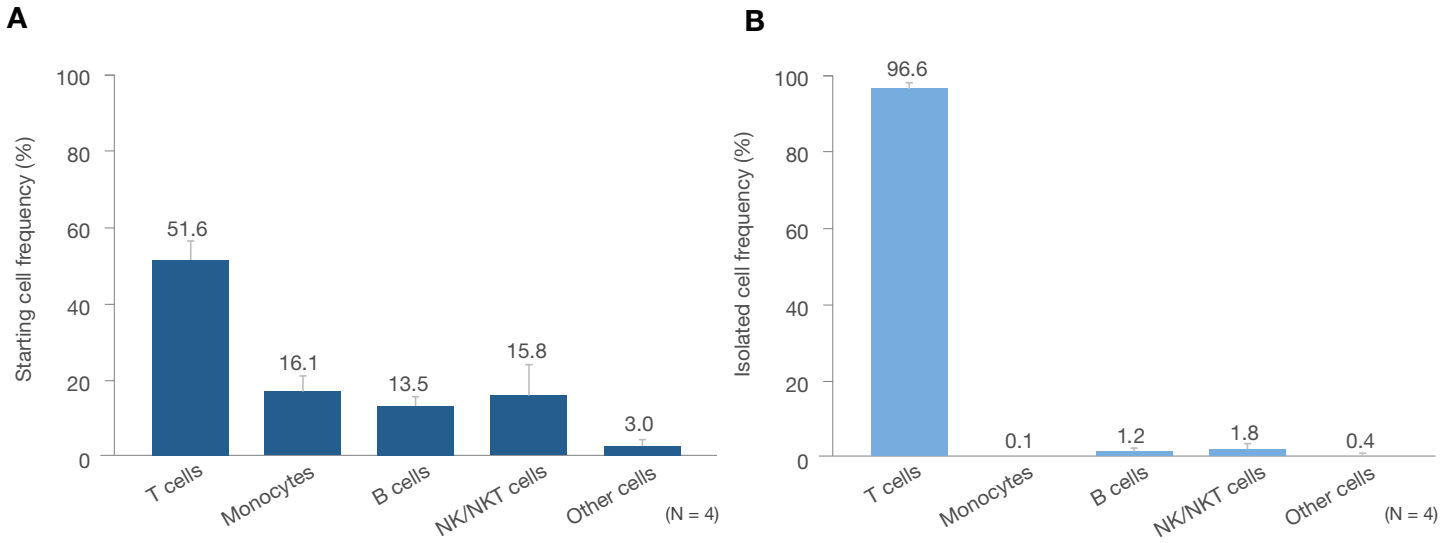


Figure 3. Target T cells are isolated at high yield with the CTS DynaCellect system and CTS Dynabeads CD3/CD28. (A) A 52% combined average frequency of the desired target T cells was determined in the starting material from four healthy donors. **(B)** An average 97% yield of the isolated target T cells was achieved by positive selection with the system and CD3/CD28 beads. (Cell frequency results were obtained using flow cytometry. The values shown are the mean frequencies of the parent population gated on viable cells from four independent experiments.)

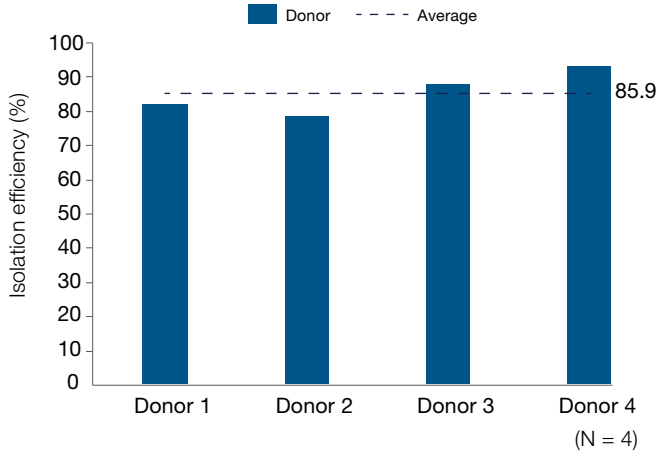


Figure 4. T cells are isolated with robust efficiency is obtained with the CTS DynaCellect system and CTS Dynabeads CD3/CD28. An 86% average (79%–94%) isolation efficiency was demonstrated with four healthy donors. (Isolation efficiency was determined by flow cytometry and cell counting of $CD3^+$ $CD28^+$ cells.)

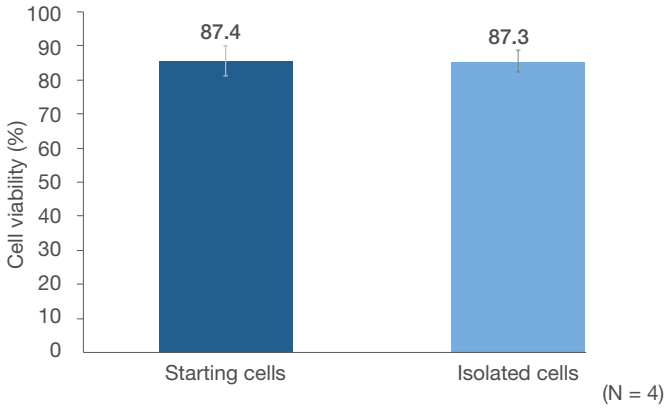


Figure 5. High viability is maintained in the isolation workflow with the CTS DynaCellect system and CTS Dynabeads CD3/CD28. An average 87% viability was maintained from the starting cells to the isolated target cell population of the four healthy donors' cells. (Cell viability was assessed by flow cytometry. The values shown are the mean frequencies of the parent population gated on viable cells.)

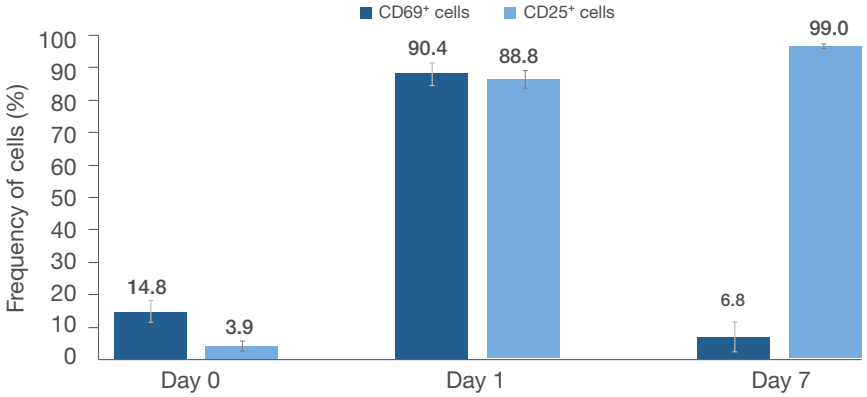


Figure 6. The CTS DynaCellect system and CTS Dynabeads CD3/CD28 support effective T cell activation and high cell expansion. The CD69 and CD25 marker expression profiles conformed to the expected target cell kinetics. The CD69 early activation marker was expressed at 90% on target cells on day 1 and then downregulated to 7% by day 7. The CD25 late activation marker was expressed at 89% by day 1 and continued to be upregulated and expressed at 99% by day 7. After isolation, evaluation of cell growth with two donor cell populations demonstrated an average 88-fold T cell expansion (data not shown). T cell activation was assessed by flow cytometry. Average frequency bars represent the mean frequencies of the parent population gated on viable cells, from four donors. Expansion was assessed in a G-Rex™ 1.0 L vessel.

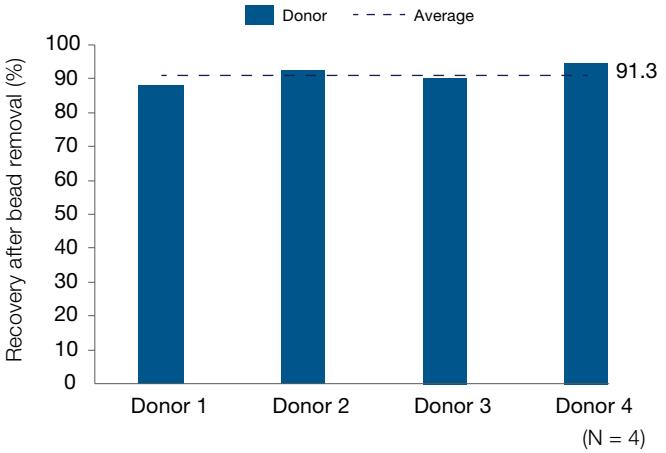


Figure 7. The CTS DynaCellect system enables high-efficiency recovery of target cells. A combined 91% average target cell recovery was demonstrated for the four healthy donors' cells, with individual recovery results ranging from 87% to 95%.

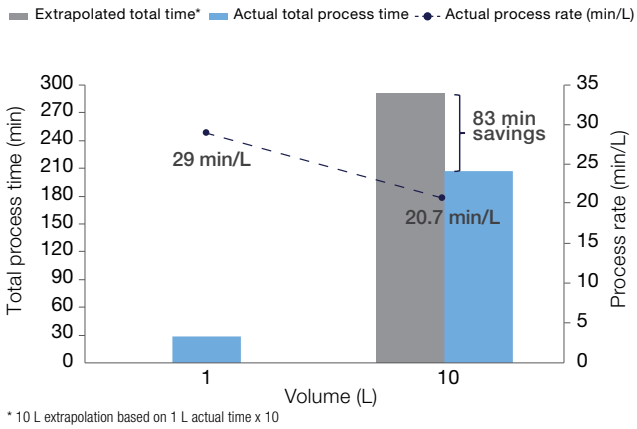


Figure 8. Cell recovery time savings can be realized with scale-up in the CTS DynaCellect system. The bead removal process rate is reduced from 29 min/L at a 1 L volume to 20.7 min/L at 10 L, resulting in a total recovery time savings of 83 min with the scale-up to 10 L.

Discussion and conclusion

The study demonstrates that the CTS DynaCellect Magnetic Separation System and single-use kits with CTS Dynabeads CD3/CD28 can provide solutions to cell therapy industry challenges. Historically, cell isolation and bead removal in cell therapy manufacturing has been performed using manual, labor-intensive, and open processes that pose risks of greater variability, human error, contamination, and imprecision. The automated and closed CTS DynaCellect system can provide the needed precision while reducing labor, variability, error, and contamination risks.

The quality and large variability of T cell starting material may result in inconsistent cell therapy processing and failure of the donor cells to reach the clinic. CTS Dynabeads CD3/CD28 were designed to isolate double-positive $CD3^+$ $CD28^+$ cells to select for the early memory T cell phenotype with stem cell–like properties. The selection of this phenotype allows the manufacturing process to be independent of the starting material quality and provides greater process standardization. The early memory phenotype has been shown to be correlated with better clinical efficacy and persistence [2].

The DynaCellect platform and CTS Dynabeads CD3/CD28 delivered up to a 97% isolation yield of target T cells, with an average 86% isolation efficiency and high cell viability. Additionally, up to 99% effective activation, 88-fold cell expansion, and 91% efficient target cell recovery were achieved. The CTS system and kits provide the necessary regulatory support documentation.

The instrument provides an integrated magnet-rocker and an eGUI fluidics panel along with features that address the needs for greater workflow flexibility, scalability, and time savings. For example, regarding flexibility, the unit is modular and can be used stand-alone or integrated with other systems using the Emerson DeltaV™ platform. It also provides the option for static or continuous processing as well as optimization of protocols. Continuous processing supports and provides the end user with a bead removal volume scale that is flexible to their needs, with potential time savings at larger volumes. To further address the demand for scalability and time savings, using CTS Dynabeads CD3/CD28 for the isolation process allows for an initial input of 10^{10} target cells to be directly processed by the DynaCellect platform in approximately 1 hr 40 min at the 1 L maximum scale.

As cell therapies continue to develop and demand increases, the CTS DynaCellect Magnetic Separation System and kits can provide manufacturers with end-to-end workflow solutions for successful commercial T cell manufacturing, essential to assuring the timely delivery of treatments for patients.

References

1. Vormittag P, Gunn R, Ghorashian S et al. (2018) A guide to manufacturing CAR T cell therapies. *Curr Opin Biotechnol* 53:164–181. **https://doi.org/10.1016/j.copbio.2018.01.025**
2. Neurauter AA, Bonyhadi M, Lien E et al. (2007) Cell isolation and expansion using Dynabeads. In: Kumar A, Galaev IY, Mattiasson B (editors), *Cell Separation. Advances in Biochemical Engineering/Biotechnology*, volume 106. Springer, Berlin, Heidelberg. **https://doi.org/10.1007/10_2007_072**

Learn more at **thermofisher.com/dynacellect**