

## Cell therapy

# CTS Detachable Dynabeads CD3/CD28 Kit provides one-step isolation and activation with flexible active release to optimize T cell phenotype

## Introduction

Nearly two decades ago, the Gibco™ CTS™ Dynabeads™ CD3/CD28 technology was used in the first pioneering, clinically approved CAR T cell therapy [1]. The antibody-coated magnetic beads were crucial in the manufacturing process to achieve one-step cell isolation and activation, and enabled clinical-scale dose production within 2 to 3 weeks. Since then, the technology has become well established in the industry and has been used in over 200 clinical trials to provide critically needed treatment for patients. Despite these successes, challenges remain to further improve the reliability, consistency, and longevity of treatment outcomes. As well, there is still a need to accelerate and expand treatment delivery to patients [2].

Research and clinical outcomes have established that delivering a higher-quality pool of early T cell phenotypes is key to improving the reliability and longevity of therapeutic outcomes [3]. To aid in the production of the desired phenotypes, the Gibco™ CTS™ Detachable Dynabeads™ CD3/CD28 Kit (active-release beads) was developed. The kit builds on the one-step isolation and activation using the CTS Dynabeads CD3/CD28 beads (passive-release beads) and provides control of activation with user-defined dissociation of the active-release beads at any time after activation. This feature was designed to help preserve an early T cell phenotype and shorten the T cell manufacturing process to a few days. Additionally, the kit is compatible for use in the scalable, closed, and automated Gibco™ CTS™ DynaCollect™ Magnetic Separation System.

The CTS Detachable Dynabeads CD3/CD28 Kit was evaluated in a comparative bench-scale study to assess the performance of T cell activation as well as post-activation cell expansion, CD4<sup>+</sup>:CD8<sup>+</sup> ratios, and early phenotype, relative to the passive-release beads. Another study was conducted with the active-release beads in the CTS DynaCollect Magnetic Separation System to evaluate T cell purity, isolation efficiency, and activation with bead release on different days post-activation.

## Materials and methods

### Comparative bench-scale study

**Cells:** Cells from frozen vials of previously isolated CD3<sup>+</sup>/CD28<sup>+</sup> T cells from 7 healthy donors were recovered in culture medium. To obtain the same starting material for activation, the T cells were negatively isolated from peripheral blood mononuclear cells (PBMCs) of healthy donors using the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit (Cat. No. 11344D). All cell counts and viability were evaluated using the Vi-CELL™ XR Cell Analyzer (Beckman Coulter).

**Medium:** Gibco™ CTS™ OpTmizer™ T-Cell Expansion SFM, no phenol red (Cat. No. A3705001), was supplemented with 2.6% Gibco™ CTS™ Immune Cell SR (ICSR, Cat. No. A2596101) and 4 mM L-glutamine. Cultures were supplemented as indicated with Gibco™ Recombinant Human IL-2 (Cat. No. PHC0023).

**Activation:** Cells were seeded at  $1 \times 10^6$  cells/mL in the medium at a total volume of 2 mL in 6-well plates. Cells were activated at a bead to T cell ratio of 3:1, with Gibco™ Dynabeads™ CD3/CD28 beads (Cat. No. 11141D) or with the CTS Detachable Dynabeads CD3/CD28 Kit (Cat. No. A56992) and 100 IU of IL-2. On day 3, the active-release beads were removed using the provided kit buffer. The passive-release beads were removed manually using an Invitrogen™ DynaMag™-2 Magnet (Cat. No.12321D).

**Expansion:** Cells were transferred on day 5 to 12-well plates and maintained through day 10 at  $0.5 \times 10^6$  cells/mL in a 2 mL volume of medium. Cell counts and viability were evaluated on days 3, 7, and 10.

**Performance criteria:** CD25 activation and the cell phenotype markers CD4, CD8, CD27, CCR7, CD62L, CD45RO, and CD3 were evaluated by flow cytometry on an Invitrogen™ Attune™ NxT Flow Cytometer with respective marker antibodies.

## Study of active-release beads in a closed, automated system

**Cells:** PBMCs from frozen leukopaks of 4 healthy donors were recovered without washing and diluted 1:1 with DPBS and 1% human serum albumin (HSA) thawing buffer. Cell counts and viability were evaluated using a NucleoCounter™ NC-3000™ system (ChemoMetec).

**Medium:** CTS OpTmizer T-Cell Expansion SFM, no phenol red, bag format (Cat. No. A3705003), was supplemented with 2.6% of the CTS OpTmizer Expansion Supplement, 2.5% CTS Immune Cell SR, 2 mM L-glutamine, 0.0125 mg/mL gentamicin, and 100,000 IU Invitrogen™ Human IL-2 Recombinant Protein (Cat. No. RP-8608).

**Isolation and activation:** On day 0,  $4.0 \times 10^8$  cells were isolated and activated at a bead to CD3<sup>+</sup> T cell ratio of 3:1, using the Gibco™ CTS™ DynaCollect™ Magnetic Separation System (Cat. No. A55867) and the Gibco™ CTS™ DynaCollect™ Isolation Kit (Cat. No. A52300). The positive and negative fractions were collected. An aliquot of the positive fraction was evaluated for day 0 cell purity. The remaining positive fraction was transferred with medium to three 1 L G-Rex™ 100M Open System vessels (Wilson Wolf). Post-activation, on each of days 1, 2, and 3, cells from one G-Rex vessel were transferred to the CTS DynaCollect Magnetic Separation System for bead release with the CTS DynaCollect Isolation Kit.

**Performance criteria:** T cell purity, CD69 and CD25 activation markers, and isolation efficiencies for CD3<sup>+</sup>/CD28<sup>+</sup> and CD3<sup>+</sup> cells were evaluated based on flow cytometry using an Attune NxT Flow Cytometer with the respective marker antibodies.

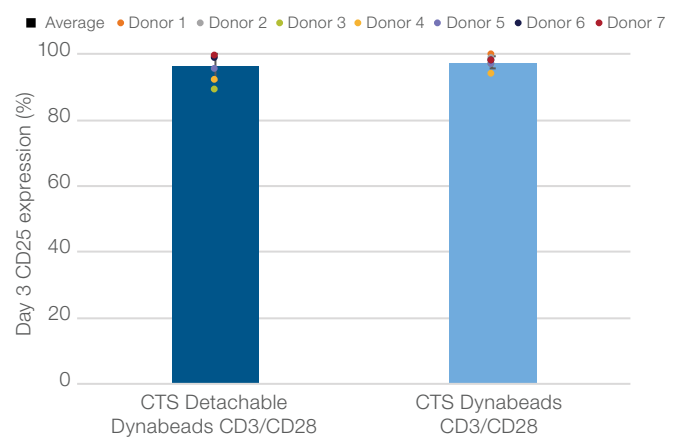
Isolation efficiency was calculated using the following equation:

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

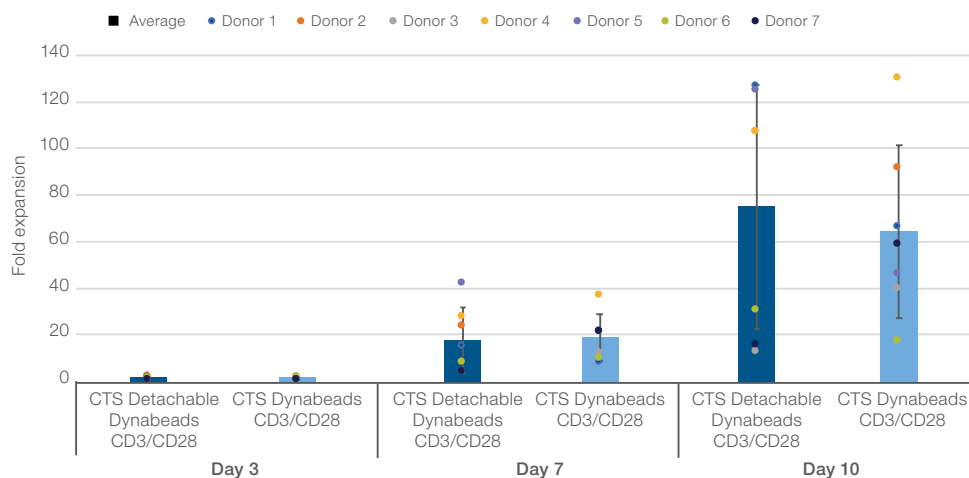
## Results

### Comparative bench-scale study

On day 3 post-activation, the average expression of the CD25 marker was >90% using the passive- and active-release beads (Figure 1). Additionally, after activation, a near 20-fold average cell expansion was shown by day 7 and near 60-fold or higher by day 10 (Figure 2). Cell viabilities remained near 90% or higher until day 10 (data not shown). The average CD4<sup>+</sup>:CD8<sup>+</sup> ratios on days 7 and 10 were comparable to those of the passive-release beads when cells were activated with CTS Detachable Dynabeads CD3/CD28 beads (Figure 3). Lastly, on day 10, cells activated with the active-release beads maintained a comparable early memory phenotype as demonstrated by the expression of CCR7<sup>+</sup>/CD62L<sup>+</sup>, CD27<sup>+</sup>/CD62L<sup>+</sup>, and CD45RO<sup>+</sup>/CD62L<sup>+</sup> marker phenotypes (Figure 4).



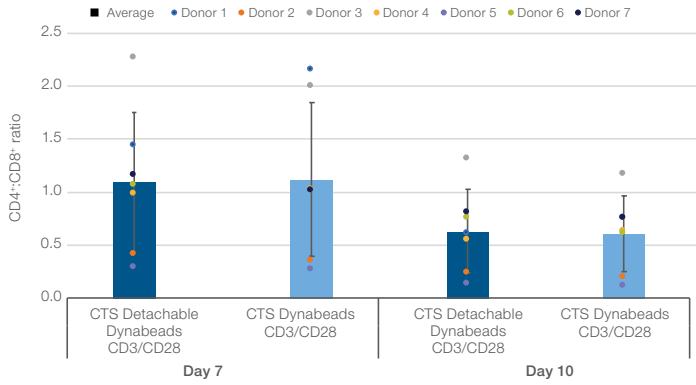
**Figure 1. Active-release beads support high T cell activation comparable to passive-release beads.** On day 3 post-activation, T cells activated with both the active- and passive-release beads show a high average frequency of cells with the CD25 activation marker, at >90%.



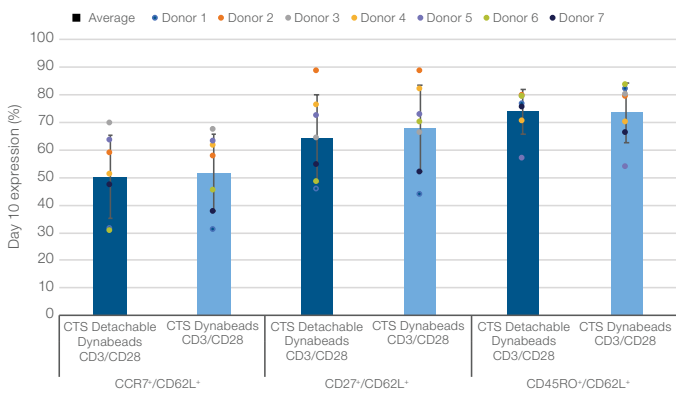
**Figure 2. Comparable cell expansion after activation with the CTS Detachable Dynabeads CD3/CD28 Kit.** Following activation with the active- and passive-release beads, T cells expanded comparably, to an average of near 20-fold by day 7 and 60-fold or higher by day 10. Cell viabilities remained near 90% or higher until day 10 (data not shown).

## Study of active-release beads in a closed, automated system

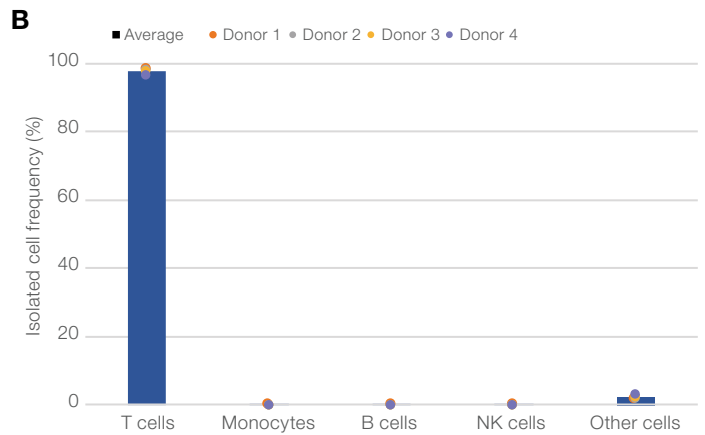
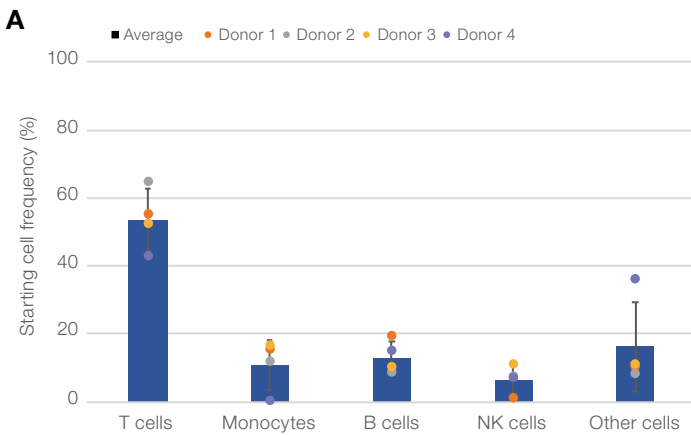
Prior to isolation, the PBMCs contained a variable mixture with an average of 54% of the desired T cell population (ranging from 43% to 65%) in combination with monocytes, B cells, NK, and other cells. The cell isolation using the CTS Detachable Dynabeads CD3/CD28 Kit in the CTS DynaCollect Magnetic Separation System produced an average T cell purity of 98% (ranging from 97% to 98%), with minimal residual cells of other types (Figure 5). The active-release beads isolated CD3<sup>+</sup> cells with an average efficiency of 80%, and more importantly, isolated the naive and early memory T cells co-expressing CD3 and CD28 with >90% efficiency (Figure 6). Evaluation of T cell activation when the beads were released on day 1, 2, or 3 demonstrated adherence of early CD69 and the later CD25 expression markers with the expected kinetics. Additionally, with the release of Dynabeads magnetic beads on day 2 or 3, nearly 100% cell activation was confirmed with an average of 94% to 98% CD25 expression (Figure 7). Cell viability was maintained at greater than 90% regardless of the day of bead release (data not shown).



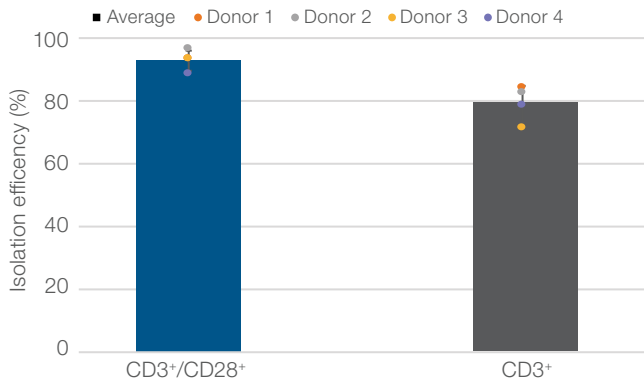
**Figure 3. CD4<sup>+</sup>:CD8<sup>+</sup> ratios are comparable with the CTS Detachable Dynabeads CD3/CD28 Kit.** The average CD4<sup>+</sup>:CD8<sup>+</sup> ratios of cells activated with the active-release beads were comparable on day 7 and day 10 to those of cells activated with the passive-release beads.



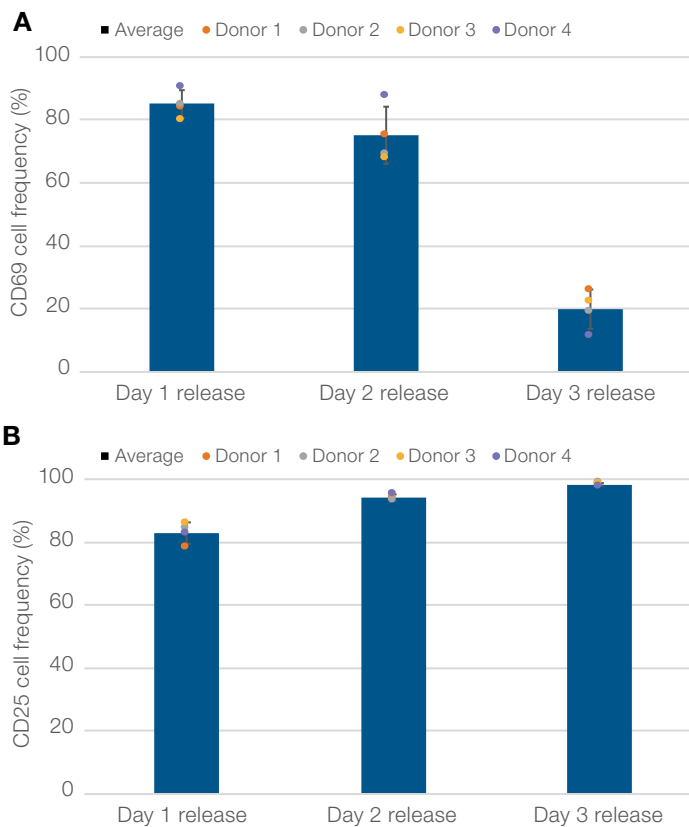
**Figure 4. Comparable T cell memory phenotype is demonstrated with the CTS Detachable Dynabeads CD3/CD28 Kit.** On day 10, cells activated with the active-release beads showed similar average expression of early phenotypes CCR7<sup>+</sup>/CD62L<sup>+</sup>, CD27<sup>+</sup>/CD62L<sup>+</sup>, and CD45RO<sup>+</sup>/CD62L<sup>+</sup>, relative to cells activated with CTS Dynabeads CD3/CD28 beads.



**Figure 5. A high target T cell purity is achieved with the CTS Detachable Dynabeads CD3/CD28 Kit with the CTS DynaCollect Magnetic Separation System.** (A) A 54% average frequency of the desired target T cell population was determined in the starting material from 4 healthy donors. (B) A high T cell average purity of 98% purity was isolated with minimal residual monocytes, B cells, and NK cells.



**Figure 6. Robust T cell isolation efficiency is obtained with the CTS Detachable Dynabeads CD3/CD28 Kit in the CTS DynaCollect Magnetic Separation System.** The active-release beads isolated CD3<sup>+</sup> cells at an average efficiency of 80%, with the naive and early memory T cells co-expressing CD3 and CD28 at >90% efficiency.



**Figure 7. The CTS Detachable Dynabeads CD3/CD28 Kit supports effective T cell activation at different time points of bead release.**

(A) The early-expressed CD69 and (B) later-expressed CD25 activation markers adhere to the expected kinetics when the beads are released on day 1, 2, or 3. On average, on days 2 and 3 near full activation of CD25 expression is observed at a range of 94%–98%. Cell viability was maintained at greater than 90% with release of beads on day 1, 2, or 3 (data not shown).

## Discussion and conclusions

The comparative study shows the CTS Detachable Dynabeads CD3/CD28 Kit performs similarly to the established passive-release beads, providing the expected one-step high-purity isolation, robust activation, cell expansion, and the desired early memory cell phenotype.

The study of active-release beads in the CTS DynaCollect Magnetic Separation System confirms the capability of the kit to deliver target T cells with high purity and robust isolation efficiency. Additionally, we showed that T cell activation reaches near full activation by day 2 or 3 post-activation. The active bead release feature provides users with the flexibility to detach the beads at any time within their process. This can allow better control of the workflow and can result in the desired early T cell phenotype. The CTS Detachable Dynabeads CD3/CD28 Kit used in combination with the CTS DynaCollect Magnetic Separation System can provide more consistent, standardized T cell production in an automated, scalable, and closed manufacturing environment.

The CTS Detachable Dynabeads CD3/CD28 Kit with the CTS DynaCollect Magnetic Separation System support the cell therapy industry's need for more flexible and robust processes that deliver consistently high-quality therapeutic products. Together, these products show strong potential to help cell therapy manufacturers provide enhanced, effective, consistent, and timely cell therapy treatment for patients.

## References

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