

EVOLVING

THE FUTURE OF CELL THERAPY MANUFACTURING: CTS DETACHABLE DYNABEADS CD3/CD28 KIT

Cell therapy



Frequently asked questions

Q. How does the Gibco™ CTS™ Detachable Dynabeads™ CD3/CD28 Kit differ from the original CTS™ Dynabeads™ CD3/CD28?

A. The CTS Detachable Dynabeads CD3/CD28 Kit features an active release mechanism for active detachment of Dynabeads magnetic beads from target T cells at any desired time. In contrast, the original CTS Dynabeads CD3/CD28 that launched to market in 2006 cannot be actively detached. They passively dissociate from cells post-isolation over time based on the binding kinetics of antibodies conjugated to their surface.

Both products possess the same Dynabeads™ M-450 microspheres at their core, but all other raw materials, such as the antibodies, are different from each other.

Q. Are CTS Dynabeads and CTS Detachable Dynabeads products for clinical trial and commercial manufacturing use?

A. Yes, CTS Dynabeads and CTS Detachable Dynabeads products are GMP-grade, which means they are eligible for use in process development, clinical trials, and commercial manufacturing.

Gibco™ Cell Therapy Systems™ (CTS™) products are GMP-compliant and include traceability documentation (such as Drug Master Files and Regulatory Support Files) for customer filing needs and a proven track record of use in many clinical trials and commercialized drugs on the market.

The CTS Dynabeads portfolio, specifically, has been actively used in over 200 clinical trials as of 2023, as well as a number of FDA-approved drugs for commercial use.

Q. How does the active release mechanism work?

A. The kit contains CTS Detachable Dynabeads magnetic beads as well as a separate bioprocess container compatible with the Gibco™ CTS™ DynaCollect™ Magnetic Separation System for automated workflows.

The bioprocess container contains an active release buffer with D-biotin that outcompetes a biotin derivative on the surface of the Dynabeads magnetic bead. The active release process is not an enzymatic reaction. After the simultaneous isolation and activation step, users may incubate the bead-bound T cells with the release buffer to actively detach the cells from the Dynabeads beads.

Users may then rely on the CTS DynaCollect system to magnetically separate the Dynabeads magnetic beads from cells, leaving a highly pure target cell population in the positive fraction.

Q. What is the recommended ratio of Dynabeads beads to target T cells?

A. When using the CTS Detachable Dynabeads CD3/CD28 Kit for simultaneous isolation and activation in one step, we recommend a 3:1 ratio of beads to target T cells. Do not calculate it based on the number of total nucleated cells in your starting material.

When the product is used strictly for activation, and isolation is performed downstream with a separate reagent, the recommended ratio is 1:1.

Q. What is the product's shelf life?

A. Multiple shelf life time-point samples were initiated simultaneously with three-lot samples from the same validation batches. At the time of the product's launch to market in 2023, the kit's shelf life is 6 months, but will be extended to 9, 12, 18, 24, 36, 48, and 60 months on a rolling basis.

Q. What are the antibodies on the surface of CTS Detachable Dynabeads CD3/CD28 beads?

A. CTS Detachable Dynabeads CD3/CD28 beads are coated with camelid-derived, single-domain (VHH) antibody fragments that identify and bind CD3 and CD28 markers separately. These antibodies are produced in a yeast-based system, virtually eliminating any risk of viral contamination.

Q. Does the use of CTS Dynabeads magnetic beads pose any potential risks to patient safety?

A. Unlike magnetic beads from other suppliers, CTS Dynabeads magnetic beads are not phagocytized by T cells or incorporated into patients. The beads can therefore be removed (with an instrument such as the CTS DynaCollect Magnetic Separation System) before the final drug is injected into the patient.

With the added benefit of an active release mechanism, CTS Detachable Dynabeads beads provide users with added assurance that their final drug product is virtually free of ancillary materials.

There is also no evidence that CTS Dynabeads magnetic beads cause nonspecific activation of T cells. For example, adding Dynabeads magnetic beads without antibodies to cell suspensions does not up-regulate activation markers or induce expansion.

Q. If the product binds to both CD3 and CD28 markers, does that mean CD3⁺ CD28⁻ cell subsets are excluded from the positive fraction?

A. Yes, the CTS Detachable Dynabeads CD3/CD28 Kit and the CTS Dynabeads CD3/CD28 preferentially isolate CD3⁺ CD28⁺ double-positive T cells, which are demonstrated to contain the clinically relevant subset of T cells (e.g., CD27⁺ PD-1⁻ CD8⁺ CAR T cells).

Also note that CTS Dynabeads magnetic beads are the only isolation technology on the market that excludes exhausted T cells from the manufacturing of the T cell drug. A study from the University of Pennsylvania and Novartis used CTS Dynabeads CD3/CD28 in a clinical study of patients with CLL [1].

Q. Are there any data or research evidence that show CD3⁺ CD28⁺ T cells are better than CD3⁺-only T cells in immunotherapy? Some researchers hypothesize that if they exclude CD3⁺CD28⁻ cells, some important subsets may be missed and the cell recovery rate is lower.

A. CD28⁻ T cells in the starting material prior to manufacturing are associated with exhausted T cells and are not relevant *in vivo* for clinical efficacy or persistence of the T cell drug.

Naive T cells require three essential signals for activation: T cell receptor (or CD3) engagement, co-stimulatory receptor (such as CD28) activation, and cytokine stimulation (IL-2 for expansion). Activated T cells down-regulate the co-stimulatory molecule CD28 and deregulate its expression altogether upon chronic antigen exposure. Down-regulation of CD28 is a hallmark of senescent T cells. CD3⁺ CD28⁻ cells are therefore activated T cells and are susceptible to activation-induced cell death (AICD) if restimulated in the absence of the co-stimulatory receptor CD28. If isolated, these cells will die during the first hours of the expansion process [2-4].

Q. What does it mean that the Dynabeads magnetic beads are superparamagnetic, and does it translate to adverse effects on my target cell suspension?

A. Superparamagnetic means that the beads exhibit magnetic properties when placed within a magnetic field but have no residual magnetism when removed from the magnetic field.

Targeted cells, proteins, or nucleic acids are subjected to magnetic forces only while the beads are on-magnet. The beads do not aggregate, and remain evenly dispersed in suspension.

Q. What is the level of support for using CTS Dynabeads products in clinical trials and commercial manufacturing?

A. CTS Dynabeads products are supported by Drug Master Files (DMFs), Regulatory Support Files (RSFs), and other traceability documentation.

For customers that enter into a Commercial Supply Agreement with us, we have dedicated resources from our quality and regulatory teams that support such customers.

Q. Can I try out CTS Detachable Dynabeads products but do so manually with columns, and not with the CTS DynaCollect Magnetic Separation System?

A. While this is suitable for assessing the functionality of the product, it does not give you the full benefits or accurate performance should you decide to scale up to clinical or commercial manufacturing.

Testing CTS Detachable Dynabeads products without a magnet such as in the CTS DynaCollect system is a highly manual, labor-intensive protocol and does not give representative yield, purity, and perhaps even activation profiles. In addition, you would need to conduct expensive bridging and comparability studies should you decide to switch to an automated magnet prior to clinical trials, because your overall process would be very different.

Q. What is the level of residual Dynabeads magnetic beads left after the bead removal step?

A. This depends on the total number of cells you are processing in a single run and the total number of beads used. However, our large-scale studies with 10 L volumes on the CTS DynaCollect Magnetic Separation System yielded less than 25 residual beads per 3 million cells. With 1 L volumes, performance was even better—we observed less than 5 residual beads per 3 million cells (in validated internal studies).

As a reference point, most regulatory agencies have an unofficial threshold of 100 residual beads per 3 million cells.

Q. What makes Dynabeads products magnetic?

A. Dynabeads beads are uniform, nonporous, superparamagnetic, monodisperse, and highly crosslinked polystyrene microspheres with an even dispersion of magnetic material throughout the bead. The magnetic material is a mixture of maghemite ($\gamma\text{-Fe}_2\text{O}_3$) and magnetite (Fe_3O_4). The iron content (Fe) of the beads is 20% by weight in Dynabeads M-450 beads.

They are also coated with a thin polystyrene shell that encases the magnetic material and prevents any leakage from the beads or trapping of ligands in the bead interior. The shell also protects the target from exposure to iron while providing a defined surface area for the adsorption or coupling of various molecules.

The uniformity of the size and shape of Dynabeads magnetic beads provides consistent physical and chemical properties. These uniform physical characteristics lead to high-quality, reproducible results.

Q. What is the benefit of this product having VHH antibody fragments for both CD3 and CD28?

A. Thermo Fisher is the only supplier that has GMP-grade reagents with this technology that can both isolate and activate T cells by a one-step mechanism. This is enabled by the CD3 and CD28 VHH antibody fragments on the surface of CTS Detachable Dynabeads CD3/CD28 beads.

The advantage is that users do not need another reagent to perform activation steps downstream from target T cell isolation. Instead, they can save process time, steps, and additional costs that would normally be required had they used separate reagents for isolation and cell activation.

Q. What is the concentration of beads in the kit?

A. The bead concentration is 4×10^8 beads/mL.

Q. What are the storage and shipping conditions for the kit?

A. We strongly recommend that you store and/or ship the product at 2–8°C protected from light. This is applicable to both the CTS Detachable Dynabeads beads provided in vials and the release buffer provided in a bioprocess container that is compatible with the CTS DynaCollect system.

Q. Are Dynabeads magnetic beads compatible with centrifugation?

A. Dynabeads magnetic beads are not designed for or intended to be used with centrifugation. Theoretically, the pressure from centrifugal forces should not be a problem for the beads themselves, because of their rigid nature and composition. However, the force exerted by the beads on cells within the pellet may be detrimental to cell viability.

Q. Are CTS Detachable Dynabeads kits compatible with any cell culture media?

A. Due to the biotin in the release buffer, we do not recommend using media with high amounts of biotin, like RPMI 1640 medium. Instead, we recommend products like Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM (no phenol red) and CTS™ OpTmizer™ Pro SFM.

Internal studies have shown that the biotin in the release buffer does not have any impact on cell phenotype or viability.

References

1. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia *Nat Med.* 2018;24(5):563-571. **doi: 10.1038/s41591-018-0010-1**
2. Huff WX, Kwon JH, Henriquez M, Fetcko K, Dey M. The evolving role of CD8+ CD28- immunosenescent T cells in cancer immunology. *Int J Mol Sci.* 2019;20(11):2810. **doi: 10.3390/ijms20112810**
3. Vallejo AN. CD28 extinction in human T cells: altered functions and the program of T-cell senescence. *Immunol Rev.* 2005;205:158-169. **doi: 10.1111/j.0105-2896.2005.00256.x**
4. Borthwick NJ, Lowdell M, Salmon M, Akbar AN. Loss of CD28 expression on CD8(+) T cells is induced by IL-2 receptor gamma chain signalling cytokines and type I IFN, and increases susceptibility to activation-induced apoptosis. *Int Immunol.* 2000;12(7):1005-1013. **doi: 10.1093/intimm/12.7.1005**

Ordering information

Product	Quantity	Cat. No.
CTS Detachable Dynabeads CD3/CD28 Kit	1 x 10 mL vial of Dynabeads magnetic beads; 400 mL release buffer	A56992
	2 x 10 mL vials of Dynabeads magnetic beads; 750 mL release buffer	A56993

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