



Boost your immunotherapeutic research

Dynabeads® *ClinExVivo*™ CD3/CD28
Dynabeads® CD3/CD28





Dynabeads® *ClinExVivo*™ CD3/CD28

- Isolate, activate, and expand your T cells *ex vivo* with just one product
- Harvest 100–1,000-fold expanded T cells in just 9–14 days
- Recover T cells with properties comparable to *in vivo*–activated T cells

Dynabeads® *ClinExVivo*™ CD3/CD28 is the well-known product formerly known as Xcyte™ Dynabeads®, co-developed by Xcyte Therapies, Inc. and Dynal Biotech AS.

The ready-to-use Dynabeads® *ClinExVivo*™ CD3/CD28 are coated with covalently linked monoclonal antibodies directed against CD3/TCR and the co-stimulatory CD28 surface receptors that are required for optimal T cell expansion. The beads are designed to expand T cells in a manner that mimics what occurs *in vivo* upon activation via antigen-presenting cells. This method eliminates the need to maintain autologous antigen-presenting cells and antigen in culture, making it the most reproducible and reliable way to stimulate T cells. The covalent attachment of antibodies to paramagnetic beads allows for easy magnetic removal of beads and antibodies after T cell expansion. For scale-up, the Dynal *ClinExVivo*™ MPC® magnet has been developed for optimal performance with Dynabeads® *ClinExVivo*™ CD3/CD28.*

Evolution of T cell–based immunotherapy

Traditional methods for expanding T cells have often been cumbersome and complex, from both technical and regulatory points

of view. Cells often required culturing for a long period of time, ultimately exhibiting compromised biological activity such as loss of key surface receptors, reduced engraftment capabilities, and limited ability to recognize a broad range of antigens.

Dynabeads® *ClinExVivo*™ CD3/CD28 have been developed to maximize *ex vivo* T cell expansion while preserving T cell viability and optimal immunobiological properties. Small- and large-scale protocols have been developed for expanding T cells (Xcellerated T Cells™) in a variety of settings. Large-scale protocols using Dynabeads® *ClinExVivo*™ CD3/CD28 were developed utilizing cost- and labor-saving bioreactor systems capable of reproducibly generating $\geq 1 \times 10^{11}$ T cells in a single culture bag or reactor in under two weeks.

Intended use

Dynabeads® CD3/CD28 and Dynabeads® *ClinExVivo*™ CD3/CD28 are intended for *ex vivo* isolation, activation, and expansion of T cells in translational research.

* In the USA, a Device Master File for Dynabeads® *ClinExVivo*™ CD3/CD28 is on file with the Food and Drug Administration, and is available for cross-referencing within an approved IND or IDE application.

Get started with preclinical research

Dynabeads® CD3/CD28 is the research-grade version of Dynabeads® *ClinExVivo*™ CD3/CD28. The two products contain the same proportion of antibodies from the same clones.

The technology has been used extensively in research studies to evaluate the use of novel adoptive T cell transfer approaches to a number of disease states, as listed in Table 1. This research includes, expansion of polyclonal T cells from peripheral blood and cord blood (1,2), viral and tumor antigen-primed T cells (3), gene-modified/transduced T cells (4,5), marrow-infiltrating tumor-specific T cells (6), and regulatory T cells (7–12). It is particularly noteworthy that bead-activated T cells are easy to gene-modify with standard gene transduction systems. This unique portfolio of T cell expansion products creates new translational research opportunities.

Clinical research applications

As listed in Table 1, the Dynabeads® *ClinExVivo*™ CD3/CD28 technology has been used in a number of clinical investigations related to various disease states.

A number of immunobiological observations have been documented:

- In stem cell transplant settings with concurrent chemotherapy-induced lymphodepletion, infusion of polyclonal bead-activated and expanded T cells resulted in early T cell recovery, with both CD4⁺ and CD8⁺ T cell counts reaching normal levels within 5–10 days post-infusion
- After infusion of bead-activated and expanded autologous T cells, a majority of chronic lymphocytic leukemia (CLL) patients experienced a significant reduction in lymphadenopathy and splenomegaly
- In a number of clinical investigations, infused T cells were long-lived, and elevated T cell counts after infusion were maintained for at least one year

Table 1—A partial list of disease states in which T cells have been effectively isolated and expanded from patients, using the Dynabeads® *ClinExVivo*™ CD3/CD28 technology.

Disease state	T cell	Type of study	Reference
Autoimmune diseases	Autologous	Preclinical	13
Chronic lymphocytic leukemia (CLL)	Autologous or allogeneic	Phase I/II	14–16
Multiple myeloma (MM)	Autologous	Phase I/II	17–19
Non-hodgkins lymphoma (NHL)	Autologous or allogeneic	Phase I/II	20–22,15
Renal cell carcinoma (RCC)	Autologous	Phase I	23
Prostate cancer (PC)	Autologous	Phase I/II	24
Chronic myeloid leukemia (CML)	Autologous	Phase I	25
HIV infection	Autologous or gene-modified T cells	Phase I/II	26–32

Note: For research use. Not intended for any animal, human therapeutic, or diagnostic use, unless otherwise stated. In the USA, a Device Master File for Dynabeads® *ClinExVivo*™ CD3/CD28 is on file with the Food and Drug Administration, and is available for cross-referencing within an approved IND or IDE application.



Additional studies using bead-activated T cells are currently underway, including:

- Gene-modified CD19-specific (scFV) T cells to treat CLL
- Suicide gene-modified T cells (TK) to treat GVHD associated with donor lymphocyte infusion
- Autologous tumor vaccine-primed, bead-activated lymph node T cells to treat renal cell carcinoma (RCC)
- HER-2/neu tumor-peptide, vaccine-primed T cells to treat BC

Expanded T cells retain optimal immunobiological characteristics

Expansion of your T cells with Dynabeads® *ClinExVivo*™ CD3/CD28 will:

- Preserve the broadest antigen-recognition capabilities by maintaining T cell receptor repertoire during the expansion process for polyclonal T cells
- Enhance *in vivo* survival and homing potential by maintaining surface CD28 expression while inducing key homing receptors (e.g. L-selectin) and survival molecules (Bcl-XL)
- Preserve both cytolytic and T helper functions through the expansion of both CD4⁺ and CD8⁺ T cells

- Induce expression or secretion of a wide range of key immunomodulatory molecules including surface-bound CD40 ligand, CD137 (4-1BB), and cytokines such as IL-2, IFN γ , and TNF α
- Reverse T cell anergy and restore response to antigenic or mitogenic stimulation

The Dynabeads® expansion platform is shown in Figure 1.

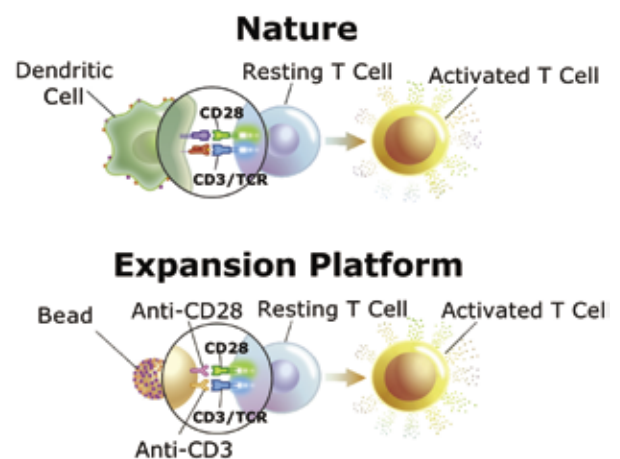


Figure 1—The Dynabeads® *ClinExVivo*™ CD3/CD28 *ex vivo* T cell expansion platform mimics *in vivo* expansion.

Scale up your research with the Dynal *ClinExVivo*[™] MPC[®] magnet

- Positively isolate bead-bound cells
- Deplete unwanted cell types
- Ideal for magnetic isolation in closed, sterile blood bags and tubing systems

The Dynal *ClinExVivo*[™] MPC[®] magnet (Figure 2) is a versatile magnetic separations device based on Dynabeads[®] technology and designed for medium- to large-scale cell separation in translational research (Figure 3).

- Scalable volumes: 50–330 ml in static separations, >10 L in continuous flow separations following T cell expansion protocols
- Residual beads that might escape initial magnetic capture are retained on a secondary magnet
- The magnetic platform can rotate 180° to optimize the capture process, reducing trapping of cells not captured by Dynabeads[®]

Intended use

The Dynal *ClinExVivo*[™] MPC[®] magnet is intended for use with the Dynabeads[®] *ClinExVivo*[™] products for translational research to positively isolate bead-bound cells:

- Positively isolate bead-bound cells (e.g., for subsequent stimulation or expansion of T cells with Dynabeads[®] *ClinExVivo*[™] CD3/CD28 and for removal of the beads following the expansion protocol).
- Deplete unwanted cell types by discarding the magnetically captured bead-bound cells (e.g. depletion of monocytes after phagocytosis of Dynabeads[®] *ClinExVivo*[™] Epoxy).

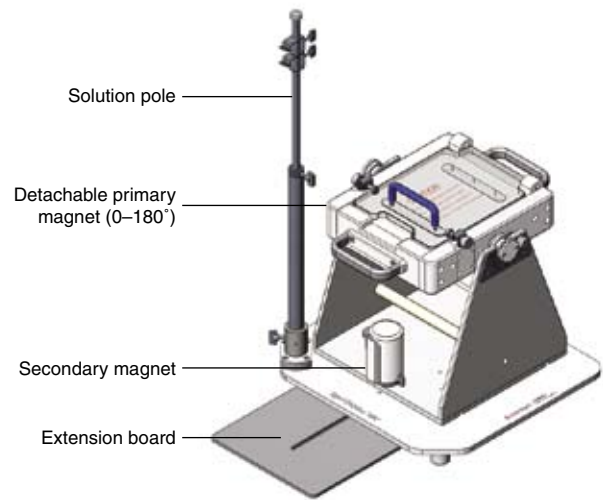


Figure 2—The Dynal *ClinExVivo*[™] MPC[®] magnet.

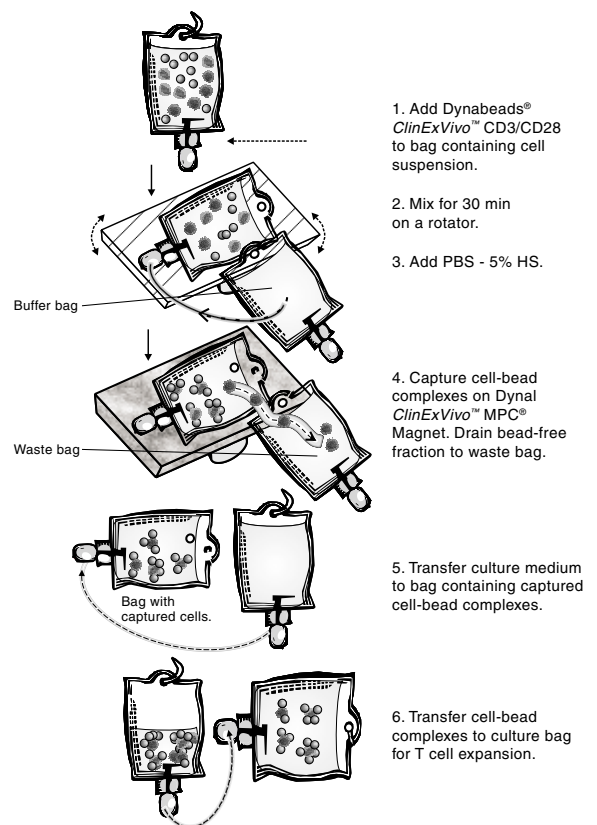


Figure 3—The Dynal *ClinExVivo*[™] MPC[®] magnet is ideal for large-scale magnetic separation.



Additional Dynabeads® *ClinExVivo*™ products—for flexible cell isolation

Two additional products are available for use with your own sterile antibodies for specific cell-type selection. Both products comply with ISO 9001:2001, ISO 13485:2003, and Medical Device Directive 93/42/EEC.

Dynabeads® *ClinExVivo*™ Sheep Anti-Mouse IgG are coated with polyclonal anti-mouse IgG antibodies. With the required IgG monoclonal antibody coupled to the beads, you can perform *ex vivo* isolation or depletion of any chosen cell type. Dynabeads® *ClinExVivo*™ Epoxy have activated epoxy groups on their surface. Unconjugated Dynabeads® *ClinExVivo*™ Epoxy can also be used to remove monocytes by direct phagocytosis of the *ClinExVivo*™ Epoxy beads.

Ordering information

Product	Quantity	Volume	Cat. no.
Dynabeads® <i>ClinExVivo</i> ™ CD3/CD28	4 × 10 ⁸ beads/ml	10 ml	402-03D
Dynabeads® CD3/CD28, research version of Dynabeads® <i>ClinExVivo</i> ™ CD3/CD28	1 × 10 ⁸ beads/ml	10 ml	111-41D
Dynabeads® <i>ClinExVivo</i> ™ Sheep anti-Mouse IgG	4 × 10 ⁸ beads/ml	10 ml	422-01
Dynabeads® <i>ClinExVivo</i> ™ Epoxy	4 × 10 ⁸ beads/ml	10 ml	402-01D
Dynal <i>ClinExVivo</i> ™ MPC®		1 unit	121-02

For current prices, please visit www.invitrogen.com.

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