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.

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

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, IFNy, and TNFα
- Reverse T cell anergy and restore response to antigenic or mitogenic stimulation

The Dynabeads® expansion platform is shown in Figure 1.
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.**

1. Add Dynabeads® *ClinExVivo™* CD3/CD28 to bag containing cell suspension.
2. Mix for 30 min on a rotator.
3. Add PBS - 5% HS.
5. Transfer culture medium to bag containing captured cell-bead complexes.
6. Transfer cell-bead complexes to culture bag for T cell expansion.

**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

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Volume</th>
<th>Cat. no.</th>
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<tr>
<td>Dynabeads® ClinExVivo™ CD3/CD28</td>
<td>4 x 10^8 beads/ml</td>
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<td>Dynabeads® CD3/CD28, research version of Dynabeads® ClinExVivo™ CD3/CD28</td>
<td>1 x 10^8 beads/ml</td>
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<td>Dynabeads® ClinExVivo™ Sheep anti-Mouse IgG</td>
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<tr>
<td>Dynabeads® ClinExVivo™ Epoxy</td>
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For current prices, please visit www.invitrogen.com.
References


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