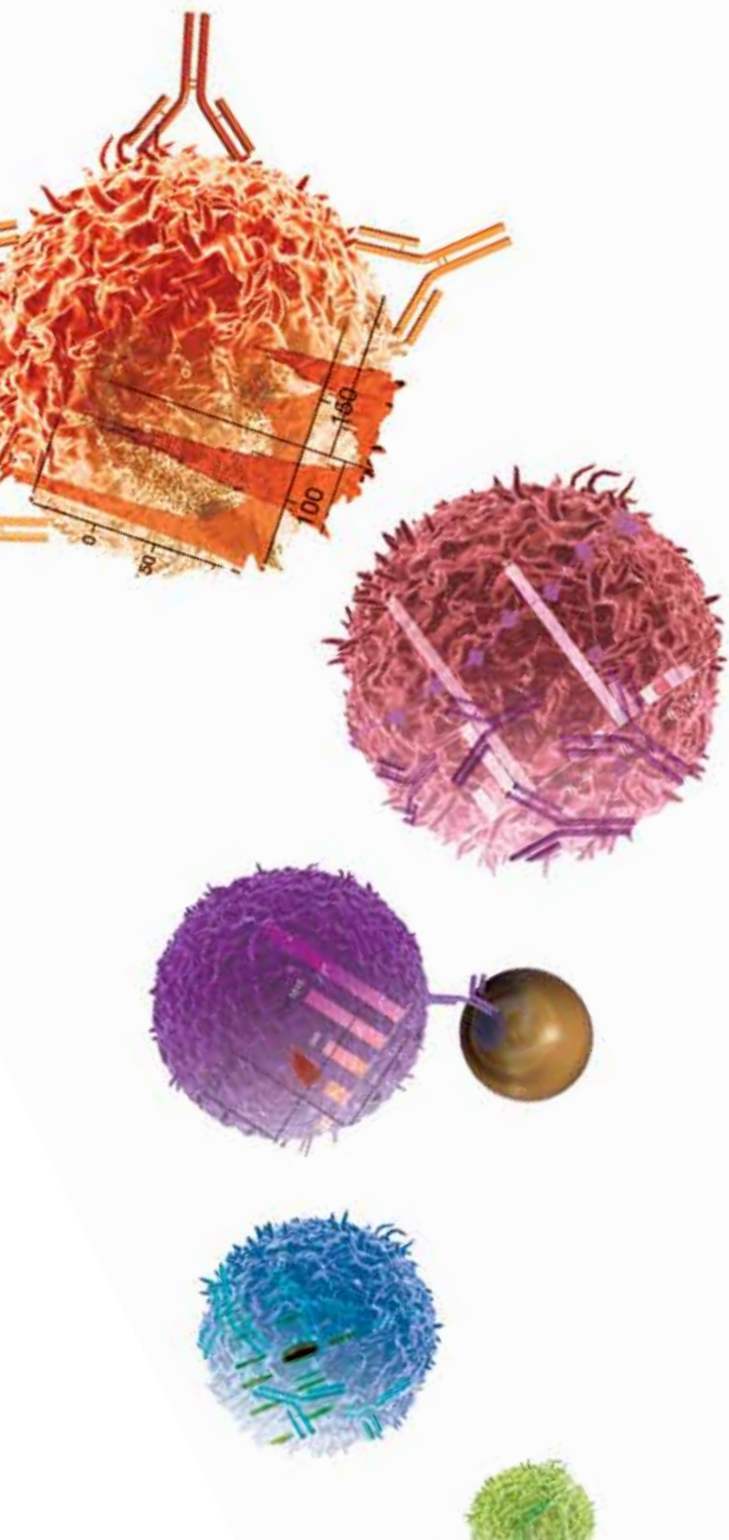


DYNAL®

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Cell Isolation and Activation



Healthy cells in = good data out

Product guide

 invitrogen™



Dynabeads® magnetic cell separation technology

- Fast, easy-to-use kits
- Gentle on cells—no mechanical stress from columns or iron exposure
- Consistent high purity and yield

Invitrogen is dedicated to delivering thoroughly tested and high-quality products that do not adversely affect cells during isolation. Dynabeads® magnetic separation technology can be used to isolate pure, viable, and functional cells of the immune system to advance your immunology research. With this technology, your cells are not exposed to the stress of passing through a dense column, so there's no risk of artifacts caused by the isolation method.

Why Dynabeads® tube-based cell separation?

When cells are removed from their natural environment, there is a risk that experimental procedures will negatively impact cell phenotype and function. The mechanical stress of being passed through a dense column matrix, as well as the exposure to iron oxide and dextran from biodegradable particles (known to be present in other isolation methods), may increase the risk of cell activation or cell death. Choosing the right cell separation method is therefore critical to your downstream experiments.

Dynabeads® are designed with an inert coating to eliminate the risk that any unwanted material such as iron is left in the sample after separation. In addition, since the cells are released from the beads after the gentle magnetic separation, high purity and viability of the cells are obtained with no artificial remnants that could affect your downstream results.

Unparalleled reproducibility and quality

In studies where patient material is scarce, difficult to obtain, or collected over a long period of time, it is critical that you can depend on the quality and reproducibility of your cell isolation method. Dynabeads® have unmatched batch-to-batch consistency, so your results are reproducible every time.

Dynabeads® tube-based cell separation technology is available in a variety of formats to suit your experimental needs: Dynabeads® flow-compatible positive isolation kits, Dynabeads® for depletion/negative isolation of cells from your sample, and secondary-coated Dynabeads® for coupling with your own antibodies. By combining our products, rare cell populations or cell subsets can be isolated. For protocols on how to optimally combine our kits, contact Technical Support or visit our website, www.invitrogen.com/cellseparation.

What are Dynabeads®?

Dynabeads® are superparamagnetic, monosized polymer beads coated with a thin polymer shell to encase the magnetic material. This specific and defined surface is ideal for the adsorption or coupling of various bioreactive molecules (ligands). The unique batch-to-batch reproducibility of Dynabeads® secures the quality of your results.

The advantages of Dynabeads® biomagnetic separation technology can be applied in a variety of manual and automated protocols for research in proteomics, protein isolation, immunology, and cellular and molecular biology, as well as diagnostic applications.



Products for cell separation

Negative and positive isolation kits and depletion products are designed for isolating cells prior to downstream cell-based assays and phenotyping. Since there are no beads left on the cells after isolation, the kits are fully compatible with flow cytometry-based applications (Figure 1).

Negative isolation kits

Negative isolation (Untouched™) kits use Dynabeads® in combination with an antibody cocktail to remove all unwanted cells from your sample, leaving only the target cells of interest behind. These kits are optimal for the isolation of fragile cells or when there are concerns that activation via antibody binding can interfere with downstream assays. Our antibody cocktails have been optimized to give you high cell purity and yield, and your resulting cells are both antibody- and bead-free.

Positive isolation kits

Dynal® positive isolation kits and FlowComp™ kits use Dynabeads® to isolate cells of interest directly from your sample (e.g., human or mouse whole blood, bone marrow or peripheral blood mononuclear cells, spleen, or lymph nodes) by binding to antibody-coated Dynabeads®. After a simple step in which the beads are removed, the cells can be used directly in all downstream experiments, including flow cytometry (Figure 2). Positive isolation is ideal when cells of the highest purity and viability are required. Our products are ideal for all types of sample material and are particularly suited for isolation of cells from viscous materials such as whole blood.

Depletion products

Dynabeads® depletion products are ideal for removal of one unwanted cell type at a time from your sample. Dynabeads® are precoated with an antibody toward one target cell type that can be depleted from all types of starting material. Our products are particularly suited for removal of cells from viscous materials such as whole blood.

Isolation of cells for molecular studies

Dynabeads® precoated with an antibody toward one target cell type can also be used for positive cell isolation when the downstream application includes molecular studies. Lyse the cells directly after isolation, and isolate proteins, DNA, or mRNA to be used in PCR, microarrays, proteomics, and other applications where the removal of Dynabeads® is not required. Target cells may be isolated from all types of starting material. These products are particularly suited for the isolation of cells from viscous materials such as whole blood.

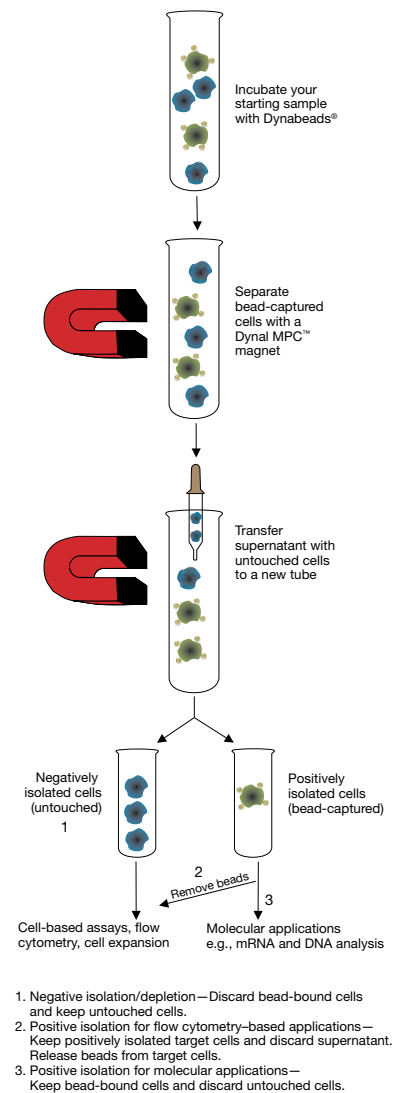


Figure 1. The workflow using Dynabeads® for positive or negative tube-based cell isolation.

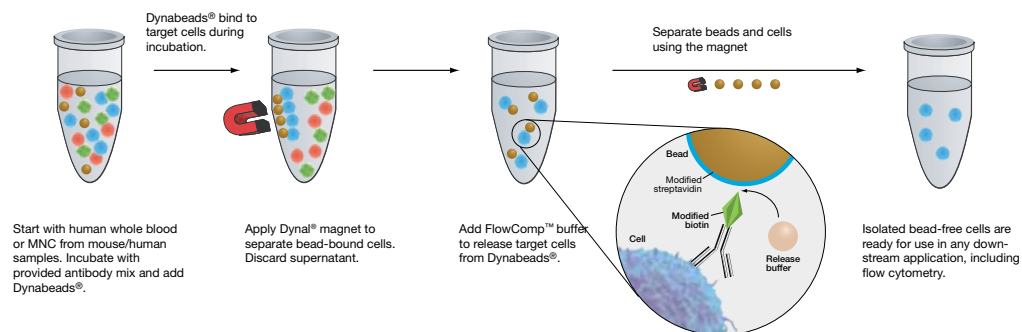


Figure 2. Overview of the Dynabeads® FlowComp™ isolation procedure. The starting sample is mouse or human mononuclear cells (MNC) or human whole blood.



Ordering information

| Isolation of human cells | | | | | |
|--|--|---------------------|----------------------|-----------|--------------------|
| Subset | Product | Application | # of cells processed | Quantity | Cat. No. |
| T cells | | | | | |
| Pan | Dynabeads® Untouched™ Human T Cells | N, Flow | 1 x 10 ⁹ | 2 x 5 mL | 113-44D |
| | Dynabeads® FlowComp™ Human CD45RA | WB, Pd, Flow | 2 x 10 ⁹ | 3 mL | 113-68D NEW |
| CD2 ⁺ T cells | Dynabeads® CD2 Pan T | WB, MA, D | 2 x 10 ⁹ | 5 mL | 111-59D |
| CD3 ⁺ T cells | Dynabeads® CD3 | WB, MA, D | 2 x 10 ⁹ | 5 mL | 111-51D |
| | Dynabeads® FlowComp™ Human CD3 | WB, Pd, Flow | 2 x 10 ⁹ | 3 mL | 113-65D |
| CD4 ⁺ T cells | Dynabeads® CD4 | WB, MA, D | 2 x 10 ⁹ | 5 mL | 111-45D |
| | Dynal® CD4 Positive Isolation Kit | WB, Pd, Flow | 2 x 10 ⁹ | 5 mL | 113-31D |
| | Dynabeads® FlowComp™ Human CD4 | WB, Pd, Flow | 2 x 10 ⁹ | 3 mL | 113-61D |
| | Dynabeads® Untouched™ Human CD4 T Cells | N, Flow | 1 x 10 ⁹ | 2 x 5 mL | 113-46D |
| CD8 ⁺ T cells | Dynabeads® CD8 | WB, MA, D | 2 x 10 ⁹ | 5 mL | 111-47D |
| | Dynal® CD8 Positive Isolation Kit | WB, Pd, Flow | 2 x 10 ⁹ | 5 mL | 113-33D |
| | Dynabeads® FlowComp™ Human CD8 | WB, Pd, Flow | 2 x 10 ⁹ | 3 mL | 113-62D |
| | Dynabeads® Untouched™ Human CD8 T Cells | N, Flow | 1 x 10 ⁹ | 2 x 5 mL | 113-48D |
| CD25 ⁺ T cells | Dynabeads® CD25* | WB, MA, D | 5 x 10 ⁹ | 5 mL | 111-57D |
| Treg cells | Dynabeads® Regulatory Human CD4 ⁺ CD25 ⁺ T Cells | Pd, Flow | 1 x 10 ⁹ | 10 mL | 113-63D |
| * Can also be used for other cells expressing CD25, such as B cells. | | | | | |
| B cells | | | | | |
| Pan | Dynabeads® Untouched™ Human B Cells | N, Flow | 1 x 10 ⁹ | 2 x 5 mL | 113-51D |
| CD 19 ⁺ B cells | Dynabeads® CD19 Pan B* | WB, MA, D, Pd, Flow | 5 x 10 ⁹ | 5 mL | 111-43D |
| CD 19 ⁺ B cells | DETACHaBEAD® CD19, for use with 111-43D | | — | 5 mL | 125-06D |
| * For cell-based assays (or cultivation) when used in combination with DETACHaBEAD® CD19. | | | | | |
| NK cells | | | | | |
| | Dynabeads® Untouched™ Human NK Cells | N, Flow | 1 x 10 ⁹ | 2 x 5 mL | 113-49D |
| Monocytes | | | | | |
| | Dynabeads® CD14 | WB, MA, D | 2 x 10 ⁹ | 5 mL | 111-49D |
| | Dynabeads® FlowComp™ Human CD14 | WB, Pd, Flow | 2 x 10 ⁹ | 3 mL | 113-67D NEW |
| | Dynabeads® Untouched™ Human Monocytes | N, Flow | 1 x 10 ⁹ | 2 x 5 mL | 113-50D |
| * With additional antibodies for improved purity. | | | | | |
| Dendritic cells | | | | | |
| | Dynabeads® Human DC Enrichment Kit* | N, Flow | 2 x 10 ⁹ | 2 x 10 mL | 113-08D |
| * Using this kit, all DC subsets are present in the enriched fraction. Combine with FACS sorting to get the specific DC subpopulation of choice. Dendritic cells may also be derived from monocytes or stem cells using Dynabeads® Untouched™ Human Monocytes or the Dynal® CD34 Progenitor Cell Selection System. | | | | | |
| Tumor cells | | | | | |
| Epithelial cells | CELLlection™ Epithelial Enrich | WB, Pd, Flow | 2 x 10 ⁹ | 5 mL | 162-03 |
| Epithelial cells | Dynabeads® Epithelial Enrich | WB, MA | 4 x 10 ⁹ | 5 mL | 161-02 |
| Epithelial cells | Dynabeads® CD45* | WB, N, Flow | 5 x 10 ⁸ | 5 mL | 111-53D |
| * For circulating tumor cell enrichment using Dynabeads® CD45, this product should not be used in combination with Dynabeads® CD15. | | | | | |
| Leukocytes | | | | | |
| | Dynabeads® CD45* | WB, MA, D | 5 x 10 ⁸ | 5 mL | 111-53D |
| * To effectively deplete all leukocytes including myeloid cells, this kit should be used in combination with Dynabeads® CD15. | | | | | |

D = depletion of unwanted cells (remaining cells may be used in downstream assays such as flow cytometry); **ESC** = embryonic stem cells; **Flow** = suitable for any cell-based downstream application such as flow cytometry, functional studies, or cell culture; **MA** = positive isolation for molecular downstream applications; **N** = negative isolation ; **Pc** = positive isolation for further culture; **Pd** = positive isolation with bead release from the cells; **WB** = ideal for whole blood.

Isolation of human cells

| Subset | Product | Application | # of cells processed | Quantity | Cat. No. |
|------------------------------|--|--------------|----------------------|----------|--------------------|
| Granulocytes | | | | | |
| CD15 ⁺ cells | Dynabeads® CD15 | WB, MA, D | 2 x 10 ⁹ | 5 mL | 111-37D |
| Endothelial cells | | | | | |
| CD31 ⁺ cells | Dynabeads® CD31 Endothelial Cell | Pc, D | 2 x 10 ¹⁰ | 5 mL | 111-55D |
| Stem cells | | | | | |
| CD34 ⁺ stem cells | Dynal® CD34 Progenitor Cell Selection System | WB, Pd, Flow | 5 x 10 ⁹ | 5 mL | 113-01D |
| Embryonic stem cells | Dynabeads® SSEA-4 | ESC, Pc, D | 5 x 10 ⁸ | 5 mL | 111-60D NEW |

For isolation of other human cell types, see the "Use your own antibody" section (pPage 6).

D = depletion of unwanted cells (remaining cells may be used in downstream assays such as flow cytometry); **ESC** = embryonic stem cells; **Flow** = suitable for any cell-based downstream application such as flow cytometry, functional studies, or cell culture; **MA** = positive isolation for molecular downstream applications; **N** = negative isolation; **Pc** = positive isolation for further culture; **Pd** = positive isolation with bead release from the cells; **WB** = ideal for whole blood.

Isolation of mouse cells

| Subset | Product | Application | # of cells processed | Quantity | Cat. No. |
|--------------------------|--|------------------|----------------------|-----------|--------------------|
| T cells | | | | | |
| Pan | Dynabeads® Mouse Pan T (Thy1.2) | MA, D | 2 x 10 ⁹ | 5 mL | 114-43D |
| | Dynabeads® FlowComp™ Mouse Pan T (CD90.2) | Pd, Flow | 2 x 10 ⁹ | 3 mL | 114-65D NEW |
| | Dynal® Mouse T Cell Negative Isolation Kit | N, Flow | 1 x 10 ⁹ | 2 x 10 mL | 114-13D |
| CD4 ⁺ T cells | Dynabeads® Mouse CD4 (L3T4)* | MA, D (Pd, Flow) | 2 x 10 ⁹ | 5 mL | 114-45D |
| | DETACHaBEAD® Mouse CD4, for use with 111-45D | DETACHaBEAD® | — | 5 mL | 124-06D |
| | Dynabeads® FlowComp™ Mouse CD4 | Pd, Flow | 2 x 10 ⁹ | 3 mL | 114-61D |
| | Dynal® Mouse CD4 Negative Isolation Kit | N, Flow | 1 x 10 ⁹ | 2 x 10 mL | 114-15D |
| CD8 ⁺ T cells | Dynabeads® Mouse CD8 (Ly-2) | MA, D | 2 x 10 ⁹ | 5 mL | 114-47D |
| | Dynabeads® FlowComp™ Mouse CD8 | Pd, Flow | 2 x 10 ⁹ | 3 mL | 114-62D |
| | Dynal® Mouse CD8 Negative Isolation Kit | N, Flow | 1 x 10 ⁹ | 2 x 10 mL | 114-17D |
| Treg cells | Dynabeads® FlowComp™ Mouse CD4 ⁺ CD25 ⁺ Treg Cells | N, Pd, Flow | 1 x 10 ⁹ | 2 x 10 mL | 114-63D |

For mouse T cell expansion kits, see the "T cell activation and expansion products" section (pPage 7).

* Compatible with cell-based assays when used in combination with DETACHaBEAD® Mouse CD4.

NK cells

| | | | | | |
|--|---------------------------------|----------|---------------------|------|--------------------|
| | Dynabeads® FlowComp Mouse CD49b | Pd, Flow | 2 x 10 ⁹ | 3 mL | 114-64D NEW |
|--|---------------------------------|----------|---------------------|------|--------------------|

B cells

| | | | | | |
|-----|--|---------|---------------------|------|--------------------|
| Pan | Dynabeads® Mouse Pan B (B220) | MA, D | 2 x 10 ⁹ | 5 mL | 114-41D |
| | Dynabeads® Mouse CD43 (Untouched™ B Cells) | N, Flow | 1 x 10 ⁹ | 5 mL | 114-22D NEW |

Dendritic cells

| | | | | | |
|--|------------------------------------|---------|---------------------|------|---------|
| | Dynabeads® Mouse DC Enrichment Kit | N, Flow | 5 x 10 ⁸ | 5 mL | 114-29D |
|--|------------------------------------|---------|---------------------|------|---------|

* Using this kit, all DC subsets are present in the enriched fraction. Combine with FACS sorting to get the specific DC subpopulation of choice.

For isolation of other mouse cell types, see especially Dynabeads® Sheep Anti-Rat IgG in the "Use your own antibody" section (pPage 6).

D = depletion of unwanted cells (remaining cells may be used in downstream assays such as flow cytometry); **Flow** = suitable for any cell-based downstream application such as flow cytometry, functional studies, or cell culture; **MA** = positive isolation for molecular downstream applications; **N** = negative isolation; **Pd** = positive isolation with bead release from the cells; **WB** = ideal for whole blood.



Use your own antibody

Dynabeads® for use with your own antibody give you the flexibility to isolate any cell type of interest. We provide a wide range of products, including Dynabeads® coated with species-specific secondary antibodies, streptavidin-coated Dynabeads®, and surface-activated Dynabeads®. Your

own antibodies can also be added to the generic positive isolation kits, such as CELLlection™ and FlowComp™, for applications where bead-free cells are desired, e.g., flow cytometry.

Ordering information

Use your own antibody

| Product | Specificity | Application | # of cells processed | Quantity | Cat. No. |
|--|----------------------------------|------------------|-----------------------|----------|----------|
| Streptavidin Dynabeads® | | | | | |
| Dynabeads® FlowComp™ Flexi* | DSB-X™ biotinylate your antibody | WB, Pd, Flow | ~2 x 10 ⁹ | 3 mL | 110-61D |
| CELLlection™ Biotin Binder | Any biotinylated antibody | WB, Pd, Flow | ~2 x 10 ⁹ | 5 mL | 115-33D |
| Dynabeads® Biotin Binder | Any biotinylated antibody | WB, MA D, N Flow | ~2 x 10 ⁹ | 5 mL | 110-47 |
| * DSB-X™ biotinylation reagents are supplied in the kit. | | | | | |
| Secondary antibody-coated Dynabeads® | | | | | |
| Dynabeads® Sheep Anti-Rat IgG | All rat IgGs | WB, MA D, N Flow | ~2 x 10 ⁹ | 5 mL | 110-35 |
| Dynabeads® Pan Mouse IgG | All mouse IgGs | WB, MA D, N Flow | ~2 x 10 ⁹ | 5 mL | 110-41 |
| Dynabeads® Pan Mouse IgG | All mouse IgGs | WB, MA D, N Flow | ~1 x 10 ¹⁰ | 5 x 5 mL | 110-42 |
| CELLlection™ Pan Mouse IgG | All mouse IgGs | WB, Pd, Flow | ~2 x 10 ⁹ | 5 mL | 115-31D |
| Dynabeads® Sheep Anti-Mouse IgG | Mouse IgG1, IgG2a, and IgG2b | WB, MA D, N Flow | ~2 x 10 ⁹ | 5 mL | 110-31 |
| Dynabeads® ClinExVivo™ Sheep Anti-Mouse IgG | Mouse IgG1, IgG2a, and IgG2b | WB, CR | | 10 mL | 422-01 |
| Dynabeads® Goat Anti-Mouse IgG | All mouse IgGs | WB, MA D, N Flow | ~2 x 10 ⁹ | | 110-33 |
| Dynabeads® Rat Anti-Mouse IgM | All mouse IgM | WB, MA D, N Flow | ~2 x 10 ⁹ | 5 mL | 110-39D |
| Dynabeads® M-280 Sheep Anti-Rabbit IgG | All rabbit IgGs | WB, MA D, N Flow | | 2 mL | 112-03D |
| Dynabeads® M-280 Sheep Anti-Rabbit IgG | All rabbit IgGs | WB, MA D, N Flow | | 10 mL | 112-04D |
| Surface-activated Dynabeads® | | | | | |
| Dynabeads® M-450 Epoxy | Any purified antibody/ligand | WB, MA D | ~2 x 10 ⁹ | 5 mL | 140-11 |
| Dynabeads® M-450 Tosylactivated | Any purified antibody/ligand | WB, MA D | ~2 x 10 ⁹ | 5 mL | 140-13 |

CR = clinical research product (cGMP-grade); **D** = depletion of unwanted cells (remaining cells may be used in downstream assays such as flow cytometry); **Flow** = suitable for any cell-based downstream application such as flow cytometry, functional studies, or cell culture; **MA** = positive isolation for molecular downstream applications; **N** = negative isolation; **Pd** = positive isolation with bead release from the cells; **WB** = ideal for whole blood; **MA D** = Secondary Dynabeads® in combination with one target antibody can be used to deplete the target cell or positively isolate for molecular studies; **N Flow** = Secondary Dynabeads® in combination with several target antibodies can be used to make your own negative isolation kit where the cells labeled with Dynabeads® are discarded and the remaining untouched cells can be used in any downstream assay.

Dynabeads® for mouse and human T cell activation and expansion

Dynabeads® CD3/CD28 for polyclonal T cell activation and expansion

- No need for antigen-presenting cells (APC) or antigen
- Gentle and easy, yet efficient, robust, and highly reproducible
- Activated cells retain their functional properties
- Use the same technology for mouse and human research-grade studies and human clinical research

Mimic *in vivo* activation of T cells

The activation of naive and memory T cells is initiated through contact-dependent antigen recognition involving antigen-presenting cells (APCs) such as dendritic cells. Activation occurs through the engagement of the T cell receptor complex (TCR/CD3, signal 1) and the CD28 costimulatory molecule (signal 2) on the surface of the T cell. Both signals are required to trigger cell-mediated immunity. Triggering of signal 1 alone can lead to T cell inactivation by anergy or apoptosis.

Dynabeads® T cell activation and expansion products offer a simple solution for mimicking the *in vivo* interaction of T cells with APCs by utilizing the two activation signals, CD3 and CD28, and a three-dimensional bead similar in size to APCs. Dynabeads® CD3/CD28 can be used for both short-term activation and long-term expansion and are easily removed from culture, allowing you to analyze your cells by flow cytometry (Figure 3). Activated cells are bead- and antibody-free, and their functions, cytokine profiles, and T cell repertoires reflect those of *in vivo* activation [1].

Controllable and reproducible activation using Dynabeads®

Traditional methods for activating and expanding T cells can be cumbersome and complex, leading to experimental variability. The use of

autologous APCs requires tedious maintenance of cultures and multiple isolations of cells. In addition, undesirable signals or soluble factors from APCs can influence experimental conditions. Soluble or plate-bound antibody stimulation or methods employing mitogens such as ConA and PHA are not ideal solutions, because they are difficult to control and reproduce. In the case of soluble antibodies the stimulation is not easily removed, and with both plate-bound and soluble methods the amount of antibody binding can vary from experiment to experiment.

Dynabeads® CD3/CD28 technology is the only activation solution that allows for a controllable setting. Dynabeads® CD3/CD28 technology is designed with covalently linked anti-CD3 and anti-CD28 antibodies for simple use and easy removal (Figure 4). Several CD3/CD28 antibody clones and ratios are available for more flexibility and optimization for your particular application (Table 1). Custom conjugations are also available (pPage 11).

Dynabeads® CD3/CD28/CD137 for antigen-specific T cell activation and expansion

A new addition to the Dynabeads® activation/expansion product portfolio, Dynabeads® CD3/CD28/CD137 is specifically optimized for activation and expansion of mouse and human antigen-specific T cells. In addition to the anti-CD3 and anti-CD28 antibodies, the new beads are also coated with anti-CD137 that recognizes CD137 (4-1BB), a costimulatory molecule expressed primarily but not exclusively on activated CD8⁺ and CD4⁺ T cells. Thus, Dynabeads® precoated with monoclonal antibodies towards CD3, CD28, and CD137 specifically enhance proliferation of antigen-specific T cells. For more information, please visit www.invitrogen.com/cellexpansion.

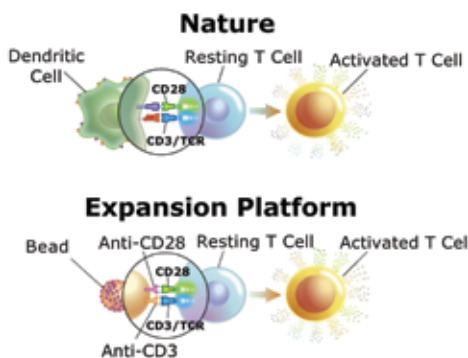


Figure 3. The Dynabeads® T cell activation/expansion platform mimics *in vivo* activation/expansion.

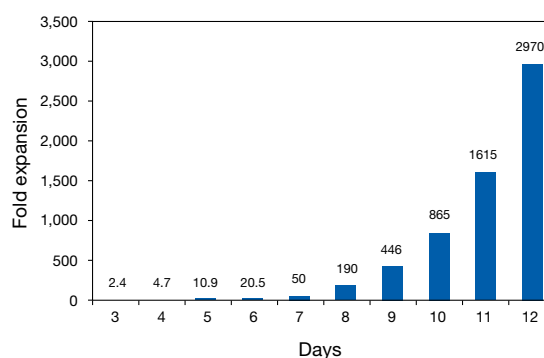


Figure 4. Expansion of naive T cells from human peripheral blood using Dynabeads® Human T-Activator CD3/CD28 for 12 days.



Ordering information

T cell activation and expansion products

| Mouse cells | Application | Quantity | Cat. No. |
|---|---------------------------------------|----------|-------------------------|
| Dynabeads® Mouse T-Activator CD3/CD28* | Polyclonal activation/expansion | 0.4 mL | 114-56D |
| Dynabeads® Mouse T-Activator CD3/CD28* | Polyclonal activation/expansion | 2 mL | 114-52D |
| Dynabeads® Mouse T-Activator CD3/CD28* | Polyclonal activation/expansion | 10 mL | 114-53D |
| Dynabeads® Mouse T-Activator CD3/CD28/CD137 | Antigen-specific activation/expansion | 0.4 mL | 114-54D NEW |
| Dynabeads® Mouse T-Activator CD3/CD28/CD137 | Antigen-specific activation/expansion | 2 mL | 114-55D NEW |
| Human cells | Application | Quantity | Cat. No. |
| Dynabeads® Human T-Activator CD3/CD28* | Polyclonal activation/expansion | 0.4 mL | 111-61D |
| Dynabeads® Human T-Activator CD3/CD28* | Polyclonal activation/expansion | 2 mL | 111-31D |
| Dynabeads® Human T-Activator CD3/CD28* | Polyclonal activation/expansion | 10 mL | 111-32D |
| Dynabeads® Human T-Activator CD3/CD28/CD137 | Antigen-specific activation/expansion | 0.4 mL | 111-62D NEW |
| Dynabeads® Human T-Activator CD3/CD28/CD137 | Antigen-specific activation/expansion | 2 mL | 111-63D NEW |
| Dynabeads® Human Treg Expander Kit | Treg activation/expansion | 2 mL | 111-29D |
| Dynabeads® Human T-Expander CD3/CD28† | Polyclonal expansion | 10 mL | 111-41D |
| Dynabeads® CD3/CD28 CTS™ ‡ | Polyclonal expansion, CR | 10 mL | 402-03D NEW NAME |

* Renamed from CD3/CD28 T Cell Expander. † Research use version of 402-03D. ‡ Renamed from Dynabeads® ClinExVivo™ CD3/CD28.

Products for clinical research

T cells expanded with Dynabeads® CD3/CD28 CTS™:

- Preserve or restore a broad T cell receptor repertoire
- Enable long-term engraftment and exhibit appropriate homing properties
- Have been employed in a number of phase I/II clinical investigations

Dynabeads® CD3/CD28 CTS™ is a unique platform for T cell isolation and expansion in the development of T cell-based therapies. This technology is also available as a research-grade version designed with the same clones, and offers a comprehensive translational research platform (Table 1, and www.invitrogen.com/DynabeadsCTS).

T cells expanded with Dynabeads® CD3/CD28 CTS™ have been used in a number of research and clinical investigations in adoptive T cell therapies for various diseases (Xcellerated T Cells™) [2–12]. T cells exhibit a broad T cell receptor repertoire following polyclonal expansion. Expanded T cells appear to have enhanced *in vivo* survival and homing potential while preserving both cytolytic and T-helper functions [13].

Large-scale expansion protocols using Dynabeads® CD3/CD28 CTS™ have been developed utilizing cost- and labor-saving bioreactor systems

that are capable of generating over 1×10^{11} T cells in a single culture bag or reactor [14].

Dynabeads® CD3/CD28 CTS™ complies with ISO 9001:2000, ISO 13485:2003, and Quality System Regulation, Medical Devices, 21 CFR Part 820. In the USA, a Master File for Dynabeads® CD3/CD28 CTS™ is on file with the Food and Drug Administration and is available for cross-referencing within an approved IND application.

DynaMag™ CTS™ magnet

The DynaMag™ CTS™ magnet is ideal for T cell isolation in closed, sterile blood bags and tubing systems. This versatile magnetic device is designed for medium- to large-scale sterile cell isolation and removal of beads prior to, during, and after expansion in translational research. The DynaMag™ CTS™ magnet is intended for use with Dynabeads® CTS™ products, but can be used with all Dynabeads® cell separation products. The scale-up capacity is 50–330 mL in static systems, and >10 L in continuous-flow systems.

For regulatory documentation for these products, contact Technical Support.

Table 1. Polyclonal T cell activation/expansion product guide.

| Product | Species | Ab clones | Features | | | Recommended starting sample | | | | For activation/expansion of | | | | | Cat. No. | Notes |
|---|---------|-----------|----------------|----------------|-----------|-----------------------------------|---|--|----------------------|--|---|--|--------------------------------|---|---|-------|
| | | | Isolation | Activation | Expansion | Leukopheresis product/elutriation | MNC/PBMC from whole blood or buffy coat | CD3 ⁺ T cells, CD4 ⁺ , or CD8 ⁺ T cell subsets, Treg cells, T cell clones | Cell lines or clones | CD3 ⁺ T cells, naive T cells, activated T cells (CD4 ⁺ or CD8 ⁺) | T cell clones (CD4 ⁺ or CD8 ⁺) | Memory T cells (CD4 ⁺ or CD8 ⁺) | Treg cells (CD4 ⁺) | Ag-specific T cells | | |
| Dynabeads® Mouse T-Activator CD3/CD28 | Mouse | | ● | ● | | ● | ● | | ● | ● | ● | ○ | | 114-56D (0.4 mL) 114-52D (2 mL) 114-53D (10 mL) | Ideal for activation/expansion of mouse T cell subsets (research use) | |
| Dynabeads® Mouse T-Activator CD3/CD28/CD137 | Mouse | | | | ● | | ● | | | | | ● | | 114-54D (0.4 mL) 114-55D (2 mL) | Efficient expansion and increased survival rate of Ag-specific T cells | |
| Dynabeads® Human T-Activator CD3/CD28 | Human | | ● | ● | | ● | ● | | ● | ● | ● | ● | | 111-61D (0.4 mL) 111-31D (2 mL) 111-32D (10 mL) | Ideal for activation/expansion of antigen-specific human T cells (research use) | |
| Dynabeads® Human Treg Expander | Human | | ● | ● | | ● | | | | | ● | | | 111-29D (2 mL) | Optimized to retain suppressive phenotype and capacity in expansion of Treg cells | |
| Dynabeads® Human T-Activator CD3/CD28/CD137 | Human | | | ● | | | ● | | | | | ● | | 111-62D (0.4 mL) 111-63D (2 mL) | Efficient expansion and increased survival rate of Ag-specific T cells | |
| Dynabeads® Human T-Expander CD3/CD28 | Human | | ● ¹ | ● ² | ● | ● | ● | ● | ● ^{3,4,5} | ● ⁶ | ● ⁷ | | | 111-41D (10 mL) | The research version of Dynabeads® <i>ClinExVivo</i> ™ CD3/CD28; intended for preclinical research applications | |
| Dynabeads® CD3/CD28 CTS™ | Human | | ● ¹ | ● ² | ● | ● | ● | | ● ^{3,4,5} | ● ⁶ | ● ⁷ | | | 402-03D (10 mL) | Intended for clinical research applications | |

● See product package insert or visit www.invitrogen.com/cell-expansion for protocols and references.
○ Unpublished data.

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Ordering information

Clinical research products

| Product | Application | Quantity | Cat. No. |
|--|--|----------|-----------|
| Dynabeads® CD3/CD28 CTS™ | T cell isolation [1] and expansion, CR | 10 mL | 402-03D |
| Dynabeads® <i>ClinExVivo</i> ™ Sheep Anti-Mouse IgG* | Cell isolation/depletion, CR | 10 mL | 422-01 |
| DynaMag™ CTS™ Magnet† | For use with blood bags, CR | <330 mL | 121-02 |
| OpTmizer™ T Cell Expansion SFM (bottle kit) | T cell expansion medium, CR | 1 L | A1048501 |
| OpTmizer™ T Cell Expansion SFM (bag kit) | T cell expansion medium, CR | 1 L | 00800225C |

1. *J Immunol* 174:2366 (2005).

*Combine with your own mouse IgG antibody (GMP-grade). †Renamed from Dynal® *ClinExVivo*™ MPC™. Scalable volumes: 50–330 mL in static separations, >10 L in continuous-flow separations. CR = clinical research product (cGMP-grade).



CD4⁺ cell counting

The Dynal® T4 Quant Kit is designed for cost-efficient and reliable quantification of CD4⁺ T cells directly from whole blood. Cell isolation takes place at room temperature in 30 minutes—with no need for sophisticated equipment and calibration curves. Cells are easily counted with a microscope, and the results are presented as the number of CD4⁺ T cells

per microliter of whole blood. A range of products are recommended for use with the Dynal® T4 Quant Kit, including staining solutions, buffer, tubes, and a hemocytometer (Figure 5). This kit is designed for monitoring immunodeficiencies, including HIV infection, and has been used successfully in developing countries. Learn more at www.invitrogen.com/t4quant.

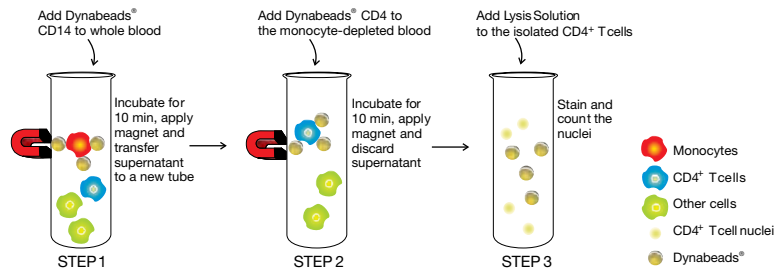


Figure 5. The Dynabeads® T4 Quant isolation procedure is fast, robust, and easily performed in three main steps. (1) Depletion of monocytes with Dynabeads® CD14. (2) Positive isolation of CD4⁺ T cells with Dynabeads® CD4. (3) The lysis solution releases the nuclei for staining or automatic counting.

Ordering information

CD4⁺ cell counting

| Product | Application | Quantity | Cat. No. |
|------------------|---|----------|----------|
| Dynal® T4 Quant* | CD4 ⁺ cell counting in blood, WB | 2 mL | 113-21D |

*This product is CE-marked. WB = ideal for whole blood.

Magnets and mixers

The new DynaMag™ magnets are optimized for efficient cell isolation using Dynabeads®. The DynaMag™ magnets combine a strong magnetic attraction for efficient separations with flexible and smart ergonomic designs that ensure comfortable working positions. The magnets can be used for any starting sample in combination with all types of Dynabeads®.

Ordering information

| Product | Optimal working volume | Cat. No. |
|-----------------|------------------------|----------|
| Magnets | | |
| DynaMag™-Spin | 10 µL–1.5 mL | 123-20D |
| DynaMag™-2 | 10 µL–2 mL | 123-21D |
| DynaMag™-15 | 1 mL–15 mL | 123-01D |
| DynaMag™-50 | 5 mL–50 mL | 123-02D |
| DynaMag™ CTS™ * | 50 mL–330 mL | 121-02 |

Mixers

| | | |
|---------------------------|----|---------|
| HulaMixer™ Sample Mixer † | NA | 159.20D |
|---------------------------|----|---------|

See www.invitrogen.com/magnets-selection for magnet and mixer specifications.

* Renamed from Dynal ClinExVivo™ MPC™. For use with blood bags. Scalable volumes: 50–330 mL in static separations, >10 L in continuous-flow separations. † Provided with 2 tube holder plates to accommodate various tube types (0.5 mL–50 mL).

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2. Thompson JA et al. (2003) *Clinical Cancer Research* 9:3562–3570.
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Dynabeads® CustomDesign™ products in cellular applications

Meeting your functional and regulatory needs

The Dynabeads® CustomDesign™ service allows you to take advantage of our proprietary technology and years of experience and know-how to customize the coupling of your ligand of interest to Dynabeads®. We have developed Dynabeads® customized products for a wide range of academic and industrial partners (including genomics, functional genomics, diagnostic, pharmaceutical, clinical, and therapeutic companies).

Since every ligand has unique characteristics, coupling conditions must be individually selected and optimized. We will help you create a unique product that suits your experimental needs, improves performance in your application, and meets your method development and regulatory requirements for cell isolation and expansion:

- Dedicated support you expect from a full-service provider
- Years of experience with ligand-coupling optimization and assay design
- Quality control and testing for sterility and endotoxins according to the United States Pharmacopeia
- ISO 13485 compliance in product development and production
- Procedures in compliance with EU's Medical Device Directive 93/42/EC, Vol 4 EU GMP Annex 18/Annex 1, 21 CFR part 820 QSR, 21 CFR part 210/211 as well as ISO 13408, as required

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