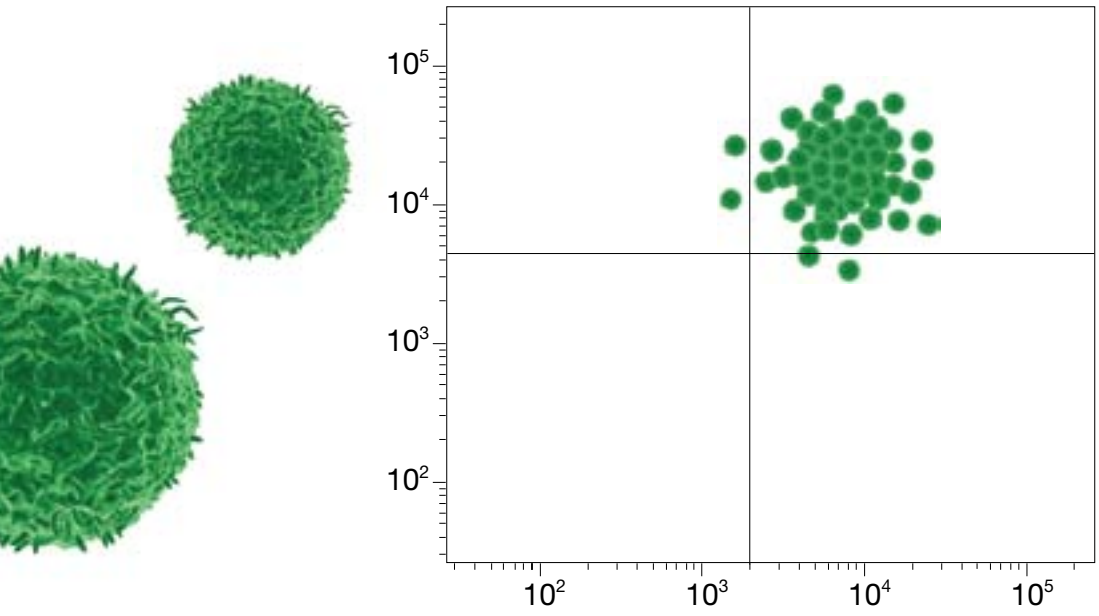
Pure and functional Treg cells

Isolate human and mouse regulatory T cells with Dynabeads®



invitrogen™

Human and mouse regulatory $CD4^+CD25^+$ T cells

Dynabeads® for gentle cell isolation

- → Isolate functional $CD4^+CD25^+$ Treg cells with high expression of Foxp3
- → Tube-based method—no columns needed
- → No beads remain bound to the cell surface
- → Isolated Treg cells can be expanded and retain their suppressive capacity and Treg phenotype

Two kits, the Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit and Dynabeads® FlowComp™ Mouse $CD4^+CD25^+$ Treg Cells, offer an easy-to-use, flow-compatible method for positive isolation of regulatory T cells (Treg cells). A simple three-step isolation procedure (Figure 1) leaves cells bead-free. The isolated cells can be directly analyzed by flow cytometry or used in any downstream experiment.

The combination of negative isolation of $CD4^+$ cells followed by positive isolation of $CD25^+$ cells secures high purity, yield, and viability of the small Treg population. You can isolate highly functional Treg cells with up to 97% purity ($CD4^+CD25^+$ expression, Figure 2); on average, more than 80% of the isolated $CD25^+$ cells express the transcription factor Foxp3.

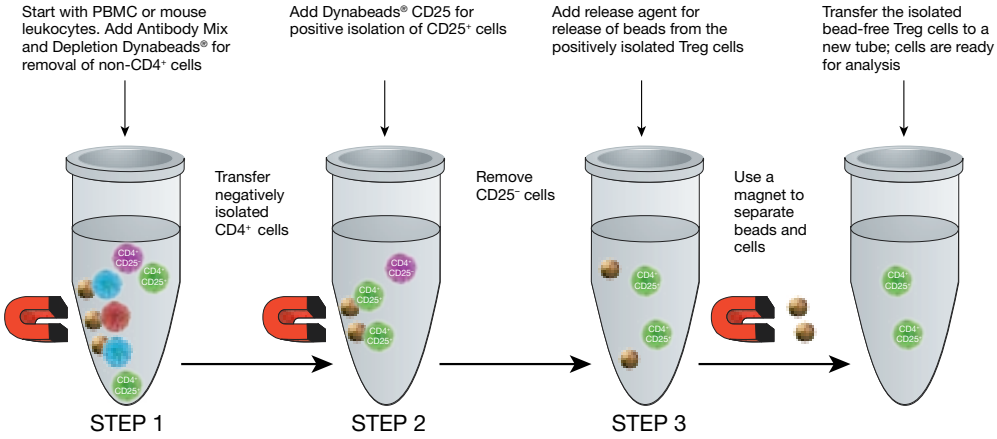


Figure 1—Three-step procedure for isolation of Treg cells. Step 1, untouched $CD4^+$ T cells are negatively isolated using Depletion Dynabeads®. Step 2, $CD25^+$ cells are positively isolated using Dynabeads® CD25. Step 3, beads are released from the $CD4^+CD25^+$ cells using the provided release reagent (FlowComp™ release buffer for the mouse product, DETACHaBEAD® release reagent for the human product. Note that DETACHaBEAD® reagent also releases the CD25 antibody from the cell surface).

Regulatory T cells

Regulatory $CD4^+CD25^+$ T cells are a specialized low-abundance subpopulation of T cells that act to maintain homeostasis within the immune system. Recently, their critical role in regulating the immune response has been firmly established. Interest in Treg cells has been accelerated by evidence from experimental mouse and human models demonstrating that the immunosuppressive potential of these cells can be utilized in the treatment of various conditions such as autoimmune diseases, infectious diseases, and cancer.

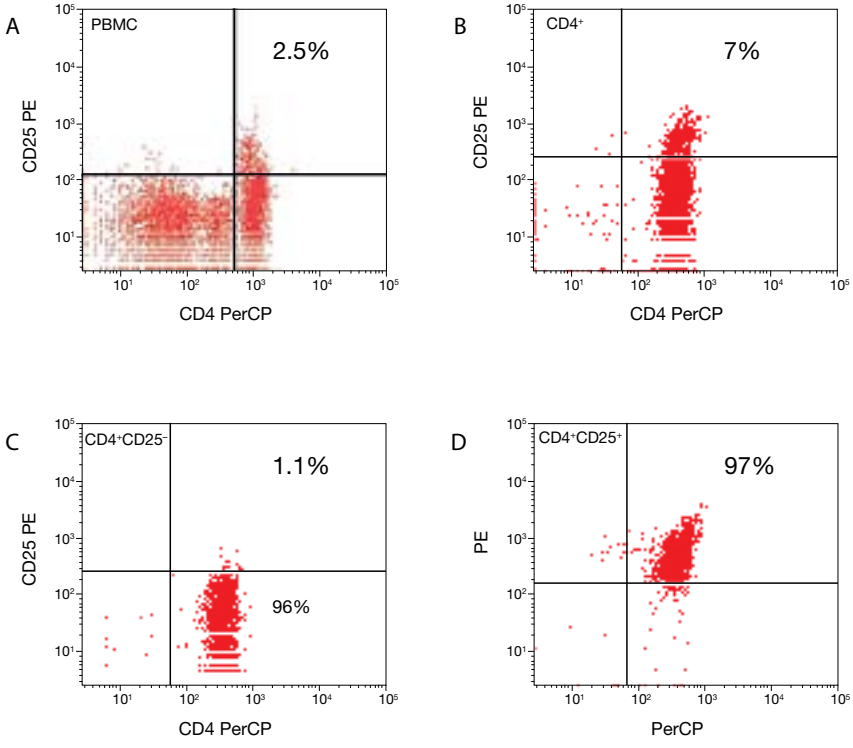


Figure 2—Presence and percentage of human $CD4^+CD25^+$ cells during the three-step isolation process using the Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit. **A.** In the PBMC sample, 2.5% of the cells were $CD4^+CD25^+$. **B.** After negative isolation of $CD4^+$ cells, 7% of the $CD4^+$ cells were $CD25^+$. **C.** After isolation of $CD25^+$ cells using Dynabeads® CD25, ~1% of the low-expressing $CD25^+$ cells remained in the $CD4^+CD25^-$ fraction. **D.** The isolated $CD4^+CD25^+$ cells were 97% pure. Similar results are seen for mouse Treg cells isolated using the Dynabeads® Mouse Regulatory $CD4^+CD25^+$ T Cell Kit (not shown).

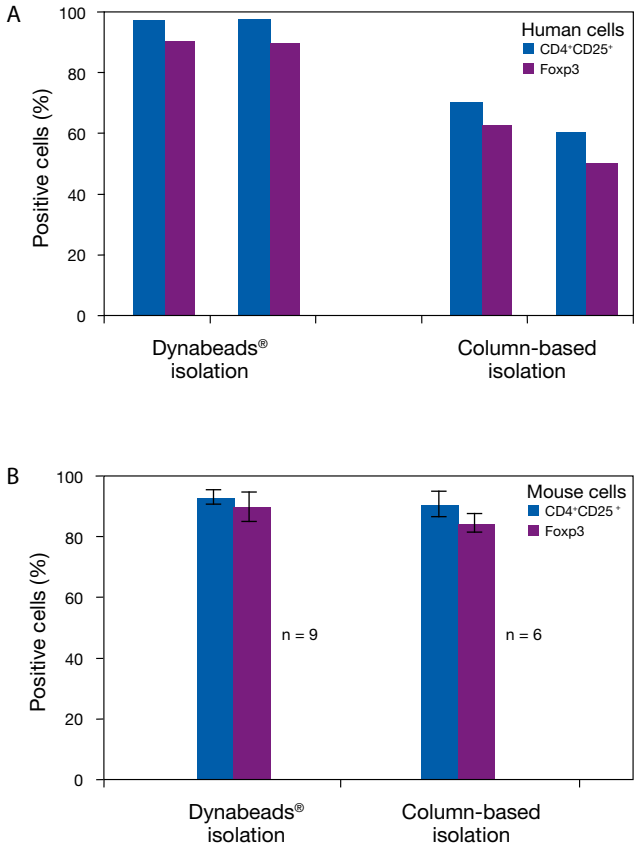


Figure 3—Higher numbers of human and mouse $CD4^+CD25^+$ $Foxp3^+$ T cells after isolation using Dynabeads®. **A.** Human Treg cells were isolated from two different donors using the Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit and a column-based method. **B.** Mouse Treg cells were isolated from mouse spleen cells using Dynabeads® FlowComp™ Mouse $CD4^+CD25^+$ Treg Cells and a column-based method. Treg purity was identified by flow cytometric analysis of CD25 and Foxp3.

Unaffected integrity of isolated Treg cells

A unique advantage of the Dynabeads® technology is the ability to isolate bead-free Treg cells. This is in contrast to alternative commercially available isolation methods in which magnetic nanoparticles are left on the surface of the cells. The uptake of such particles may be related to cytotoxic/immunogenic effects on cells, a problem recently recognized when working with biodegradable nanoparticles.[1,2]

The Dynabeads® polymer coating prevents unwanted exposure of your cells to iron oxides or dextran during isolation. Adding a release reagent separates the cells and the Dynabeads® in one simple step. The integrity of your Treg cell population is not impacted by the gentle, tube-based isolation method.

High Treg purity and functional capacity

CD25 and Foxp3 are expression markers associated with suppressive function in Treg cells. A significantly higher number of cells expressing both the CD25 and Foxp3 markers is isolated using Dynabeads® than using a column-based method (Figure 3).

The functionality of isolated $CD4^+CD25^+$ Treg cells can be examined by co-culturing them with $CD4^+CD25^-$ effector T cells. As an example, the Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit was used to isolate both the $CD4^+CD25^+$ and $CD4^+CD25^-$ human cell populations. In a suppression assay that followed, the isolated Treg cells profoundly inhibited the proliferation of CFSE-labeled $CD4^+CD25^-$ T cells (Figure 4).

Expansion of isolated Treg cells

Because of the low proportion of Treg cells in blood (2–10%), it is often necessary to expand isolated Treg cells to perform desired experiments. The Dynabeads® CD3/CD28 T cell expansion technology mimics *in vivo* T cell activation via antigen-presenting cells (APCs) and allows for gentle and efficient physiological *ex vivo* activation and expansion in both mouse and human settings.

You can obtain a 100-fold increase in the number of Treg cells (Figure 5A), and the specific stimulation and expansion may be enhanced using rapamycin.[3,4] Using Dynabeads® for isolation and expansion, you can always count on good yields of highly pure Treg cells, and avoid having to perform multiple isolations using less efficient techniques.

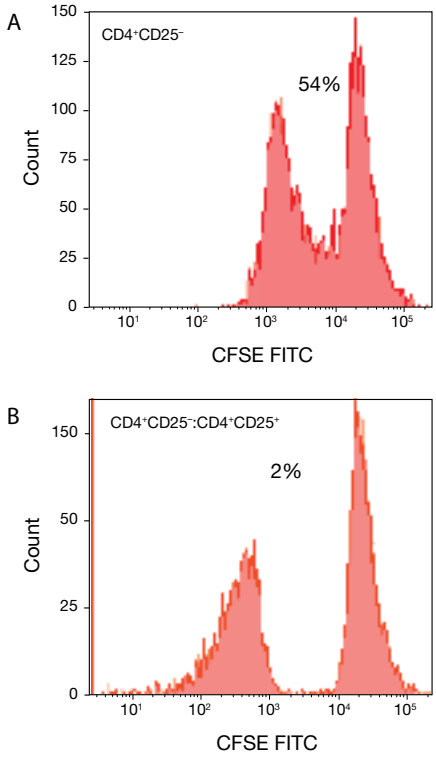


Figure 4—Suppressive capacity of isolated human Treg cells. **A.** Human $CD4^+CD25^-$ cells were CFSE-stained and stimulated with Dynabeads® CD3 (bead:cell ratio 1:1). On day 4, 54% of the cells were dividing as identified by flow cytometry. **B.** Human $CD4^+CD25^-$ cells were CFSE-stained and stimulated with Dynabeads® CD3 in the presence of human $CD4^+CD25^+$ Treg cells in a 1:1 ratio. After 4 days, only 2% of the $CD4^+CD25^-$ cells were dividing; 96% suppression of cell division was achieved in the presence of the human $CD4^+CD25^+$ Treg cells (left peak in histogram shows human Treg cells not CFSE-stained). Similar results are seen with mouse Treg cells isolated using the Dynabeads® Mouse Regulatory $CD4^+CD25^+$ T Cell Kit (not shown).

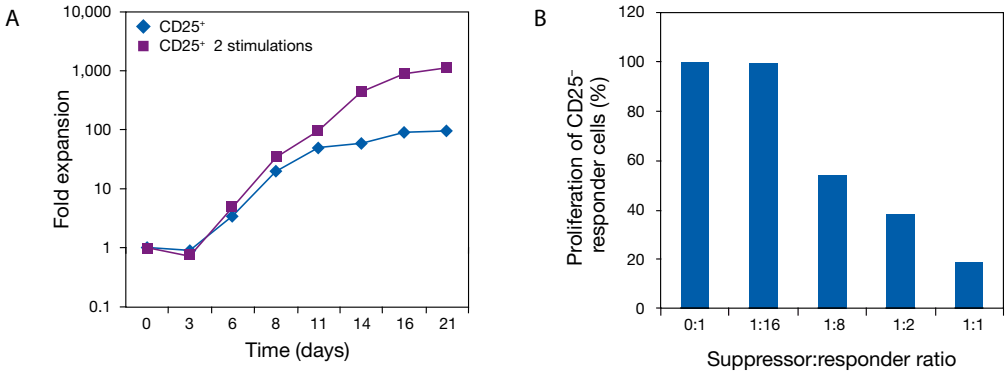


Figure 5—Treg cells activated with Dynabeads® CD3/CD28 T cell expansion technology can be expanded 100-fold while retaining their Treg phenotype and functionality. **A.** A higher expansion number can be obtained through restimulation at day 8 with >97% pure $CD4^+CD25^+$ Treg cells to avoid overgrowth of non-Treg cells. **B.** Expanded human Treg cells were co-cultured with allogenic PBMC for 6 days at various suppressor:responder ratios. Proliferation was measured using a standard thymidine incorporation assay. At a 1:1 ratio, 80% suppression was achieved.

Treg suppressive capacity is maintained

The expanded Treg cells are functional and can suppress effector T cells in a mixed lymphocyte culture (MLC) reaction (Figure 5B). The Treg cells retain their Foxp3 expression, and a higher purity is seen after both isolation and expansion using Dynabeads® than is seen with a column-based method (Figure 6).

Expanded mouse T cells can be used for further *in vitro* manipulations, or for adoptive transfer *in vivo*. Our full portfolio of activation and expansion Dynabeads® includes a clinical research–grade product, allowing you to move from mouse studies to clinical research (Phase I/II) using the same technology platform.

References

1. Pisanic, T.R., II et al. (2007) *Biomaterials* 28:2572–2581.
2. Berry, C.C. et al. (2004) *Biomaterials* 25:5405–5413.
3. Valmori, D. et al. (2006) *J Immunol* 177:944–949.
4. Battaglia, M. et al. (2006) *J Immunol* 177:8338–8347.

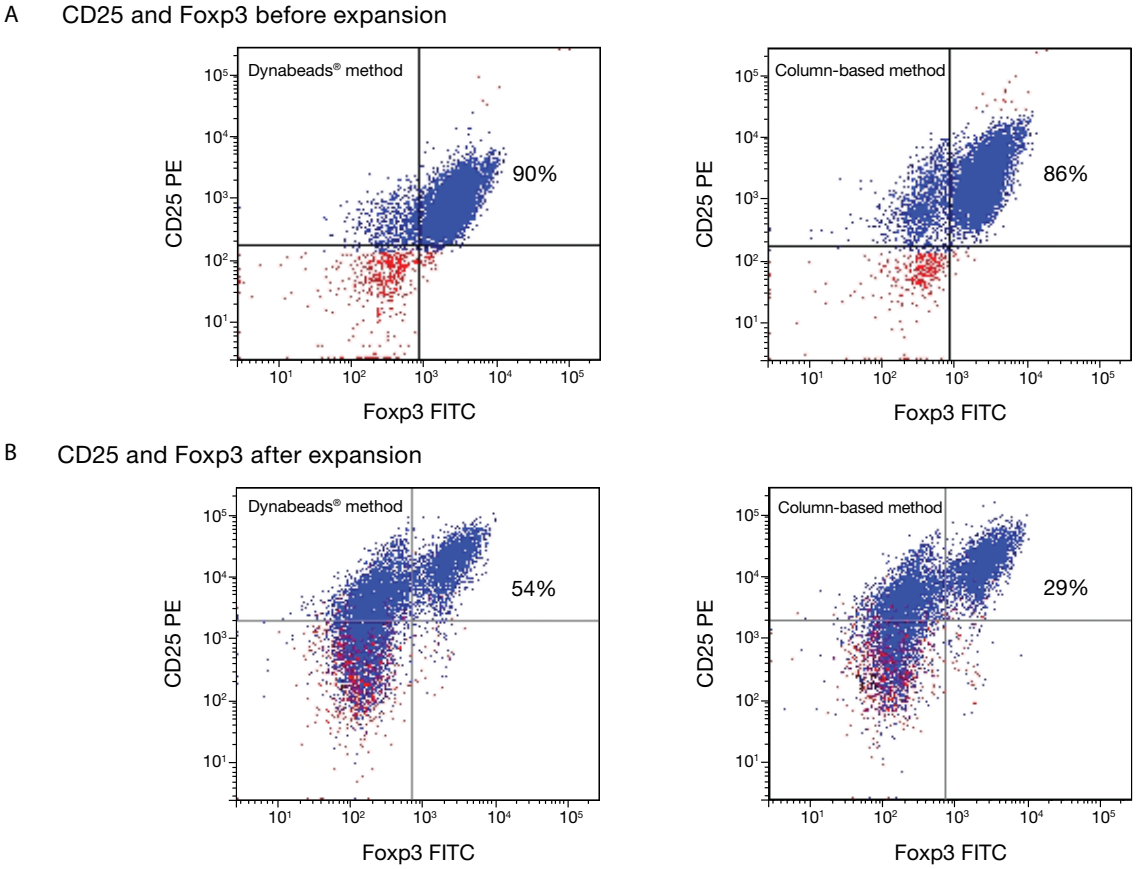


Figure 6—Expanded mouse Treg cells retain Foxp3 expression. The purity of mouse Treg cells isolated with the Dynabeads® FlowComp™ Mouse $CD4^+CD25^+$ Treg Cells kit and a column-based method, before **(A)** and after **(B)** 10 days of expansion with Dynabeads® Mouse CD3/CD28 T Cell Expander, as measured by CD25 and Foxp3 expression.

Ordering information

Product	Application	Quantity	Cat. no.
Dynabeads® Regulatory $CD4^+CD25^+$ T Cell Kit The kit contains Antibody Mix, Depletion Dynabeads®, Dynabeads® CD25, and DETACHaBEAD® release reagent	Human Treg cell isolation	Processes 1 x 10^9 cells	113-63D
Dynabeads® FlowComp™ Mouse $CD4^+CD25^+$ Treg Cells The kit contains Antibody Mix, Depletion Dynabeads®, FlowComp™ Dynabeads® (mTreg), FlowComp™ Mouse CD25 Antibody, and FlowComp™ Release Buffer	Mouse Treg cell isolation	Processes 1 x 10^9 cells	114-63D
Magnets			
DynaMag™-15 (holds 4 standard 15 ml tubes, alternatively 4 x 5 ml tubes used in flow cytometry)	Magnetic separation	1 unit	123-01D
DynaMag™-50 (holds 2 x 5–50 ml tubes)	Magnetic separation	1 unit	123-02D
See www.invitrogen.com/magnets for other magnet recommendations.			
Related human products			
Dynabeads® Untouched ™ Human CD4 T Cells	Cell isolation	Processes 1 x 10^9 cells	113-46D
Dynabeads® CD3	Cell isolation	5 ml	111-51D
Dynabeads® CD25	Cell isolation	5 ml	111-57D
Dynabeads® CD3/CD28 T Cell Expander	T cell expansion	2 ml	111-31D
Dynabeads® CD3/CD28 T Cell Expander	T cell expansion	10 ml	111-32D
Dynabeads® Human Treg Expander	Treg cell expansion	2 ml	111-29D
Dynabeads® CD3/CD28	Preclinical cell expansion	10 ml	111-41D
Dynabeads® *ClinExVivo*™ CD3/CD28	Clinical-grade cell expansion	10 ml	402-03D
Related mouse products			
Dynal® Mouse CD4 Cell Negative Isolation Kit	Cell isolation	Processes 1 x 10^9 cells	114-15D
Dynabeads® Mouse CD3/CD28 T Cell Expander	T cell expansion	2 ml	114-52D
Dynabeads® Mouse CD3/CD28 T Cell Expander	T cell expansion	10 ml	114-53D
For current prices, please visit www.invitrogen.com.			

Learn more about the best starting point for your T cell research at www.invitrogen.com/cellisolation.

Did you know that Dynabeads® magnetic separation technology was pioneered in the 1980s by the Norwegian company Dynal, now part of Invitrogen? To learn more, visit www.invitrogen.com/dynal.

DYNAL® has pioneered magnetic separation technology for biological discovery that is both simple and highly reproducible. Based on their patented superparamagnetic, monodisperse beads, Dynabeads® technologies represent a superior paradigm for cell and biomolecule separation in a wide range of basic and clinical research applications, diagnostic assays, and therapeutic protocols.

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