

Putting regulatory T cells to work

ISOLATE AND EXPAND CD4⁺ CD25⁺ TREG CELLS USING DYNABEADS[®] MAGNETIC SEPARATION TECHNOLOGY.

Defined by their functional ability to regulate or suppress immune responses, regulatory CD4⁺ CD25⁺ T cells (Treg cells) are a specialized subpopulation of T cells that act to maintain homeostasis within the immune system. Naturally occurring Treg cells (CD4⁺ CD25⁺ FOXP3⁺ T cells) have been identified in nonmanipulated rodents and humans and comprise cells of the adaptive immune system.^{1,2} In addition, it has been reported that an uncommitted T cell can be skewed toward a regulatory function (Tr1 and Th3 cells) in the presence of suppressive cytokines such as IL-10 and TGFβ.^{3,4} Treg cells are hyporesponsive to T cell receptor (TCR) stimulation *in vitro*, but exogenous IL-2 and strong costimulation with anti-CD28 can overcome their anergic state. On polyclonal or antigen-specific TCR stimulation, Treg cells potently suppress the proliferation and cytokine production of effector CD4⁺ and CD8⁺ cells by inhibiting IL-2 gene transcription. The 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 diseases such as autoimmunity, infectious diseases, and cancer.⁵⁻¹¹ Invitrogen's Dynabeads[®] Treg products provide a path from isolation to stimulation to analysis of this therapeutically relevant class of immune cells.

Capturing Treg cells from clinical samples

Treg cells can be isolated from human blood, lymphoid organs, umbilical cord blood, and the thymus, and comprise 1–10% of the total CD4⁺ T cell population in peripheral blood. Based on Dynabeads[®] technology, we have developed a new isolation and expansion protocol for human CD4⁺ CD25⁺ regulatory T cells with characteristic phenotype and suppressive capacity by using Dynabeads[®] Regulatory CD4⁺ CD25⁺

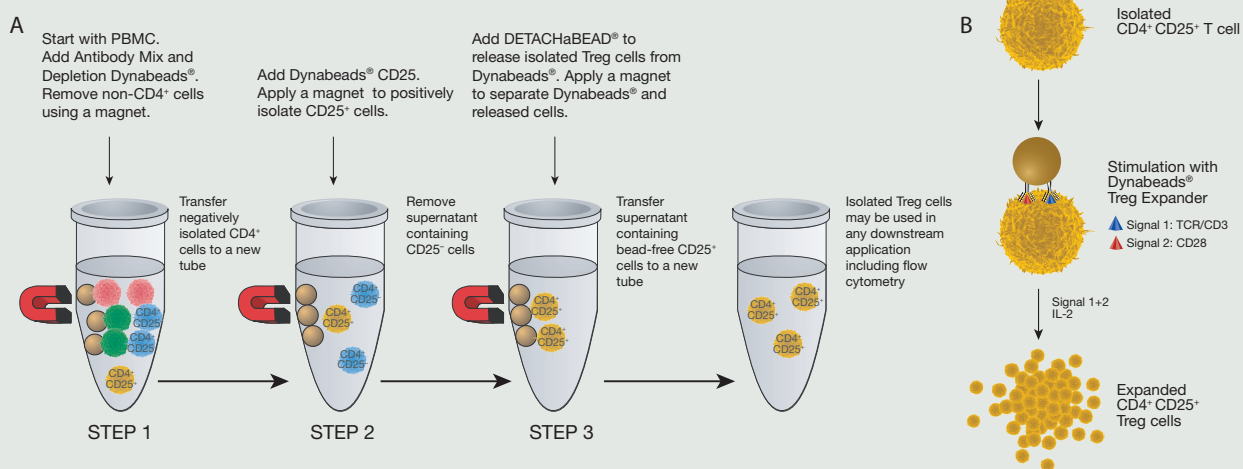


Figure 1—An overview of the isolation and expansion protocol for human Treg cells. (A) CD4⁺ CD25⁺ regulatory T cells are isolated from peripheral blood mononuclear cells (PBMC) with the Dynabeads[®] Regulatory CD4⁺ CD25⁺ T Cell Kit by negative isolation of CD4⁺ T cells followed by positive isolation with Dynabeads[®] CD25 and bead detachment with DETACHaBEAD[®] reagent. Isolated CD4⁺ CD25⁺ regulatory T cells are bead- and antibody-free. (B) Expansion of CD4⁺ CD25⁺ Treg cells using Dynabeads[®] Treg Expander.

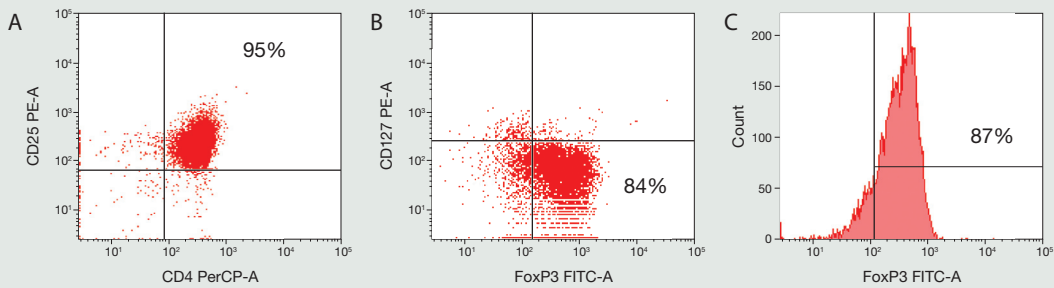


Figure 2—Treg phenotype after isolation. Isolation of Treg cells with the Dynabeads® Regulatory CD4+ CD25+ T Cell Kit. (A) The isolated Treg cells are 95% pure. (B) Within the CD4+ CD25+ Treg cell population, 84% express FoxP3 and are negative for CD127. (C) In total, 87% of the CD4+ CD25+ Treg cells express FoxP3.

T cell Kit and Dynabeads® Treg Expander (Figure 1). The unique positive isolation method allows the release of both the bead and the anti-CD25 antibody, which eliminates undesirable effects that occur when the IL-2 α chain (CD25) becomes bound by antibody.¹² It is also possible to isolate both T effector (CD4⁺ CD25⁻) and T regulatory (CD4⁺ CD25⁺) populations using this kit.

Isolated Treg cells are phenotypically correct and retain their suppressive capacity

Highly pure ($\geq 95\%$) regulatory CD4⁺ CD25⁺ T cells were isolated from the peripheral blood mononuclear cells (PBMC) of healthy blood donors using the Dynabeads® Regulatory CD4⁺ CD25⁺ T Cell Kit. A large

majority of these cells ($\geq 80\%$) expressed the FoxP3 transcription factor (Figure 2). Comparison of Dynabeads® technology with a column-based isolation technology revealed that the Dynabeads® tube-based isolation strategy resulted in a significantly higher number of CD25⁺ as well as FoxP3⁺ T cells (Figure 3).

To assay suppressive capacity, CD4⁺ CD25⁻ effector T cells were stained with CFSE (Cat. no. C34554) and mixed with CD4⁺ CD25⁺ Treg cells. Dynabeads® CD3 (1 bead/cell) were added to activate the effector CD25⁻ T cells, and suppression was measured 4 days later using CFSE staining. Treg cells suppressed the proliferation of CD4⁺ CD25⁻ effector T cells in the presence of CD3 activation by up to 96% (Figure 4), showing that the isolated Treg cells retain their normal function. →

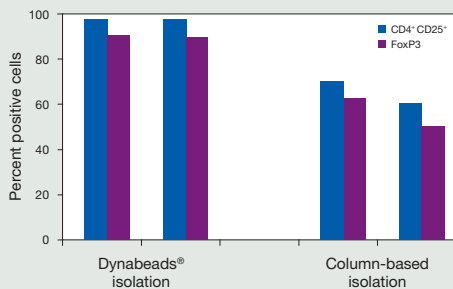


Figure 3—Comparison of the Dynabeads® tube-based isolation strategy and a column-based method. Treg cells were isolated from two different donors using the Dynabeads® Regulatory CD4+ CD25+ T Cell Kit or a column-based method. Purity was assessed by flow cytometric analysis with anti-CD25 and anti-FoxP3 antibodies.

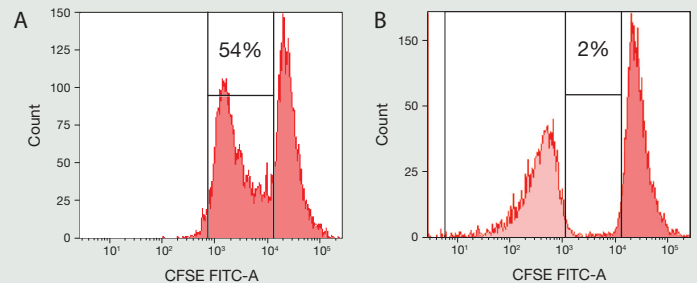


Figure 4—Suppressive capacity of isolated Treg cells. (A) CD4+ CD25⁻ cells were stained with CFSE and stimulated with Dynabeads® CD3 (1 bead/cell) for four days. On day 4, 54% of the cells were dividing as identified by flow cytometry. (B) CD4+ CD25⁻ cells stained with CFSE were stimulated with Dynabeads® CD3 in the presence of CD4+ CD25+ Treg cells in a 1:1 ratio. After four days, only 2% of the CD4+ CD25⁻ cells were dividing and 96% suppression of cell division was achieved in the presence of CD4+ CD25+ Treg cells (unstained CD4+ CD25+ cells shown in light red).

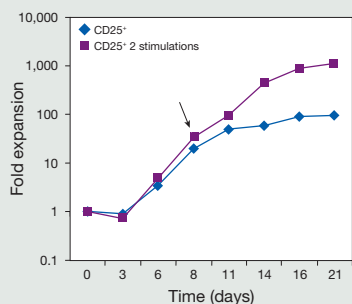


Figure 5—Expansion of isolated regulatory T cells. Highly pure Treg cells (>95%) isolated with the Dynabeads® Regulatory T Cell Kit can be expanded 100-fold while retaining their Treg phenotype with one round of stimulation using Dynabeads® Treg Expander. High initial purity of the isolated Treg cells (>97%) allows restimulation at day 8 (arrow) to obtain even higher expansion levels (1,000-fold) without overgrowth of non-Treg cells.

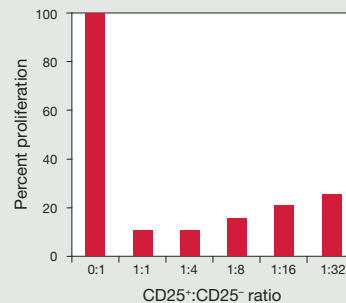


Figure 6—Suppressive capacity of rapamycin-generated Treg cells. CD4⁺ T cells expanded with Dynabeads® CD3/CD28 and rapamycin were cocultured with Dynabeads® CD3/CD28 only to evaluate the suppressive capacity of the rapamycin-expanded cells. At day 4, proliferation was analyzed in a thymidine incorporation assay. Suppression of ~80% could be maintained with a 1:32 CD25⁺:CD25⁻ ratio.

Expand the possibilities for functional studies

Low numbers of regulatory T cells can be a roadblock for scientists wishing to perform functional and/or adoptive cell transfer experiments.¹³ To expand the CD25⁺ Treg population, Dynabeads® Treg Expander reagent was added to 1 × 10⁶ cells/ml (3 beads/cell) for 14 days. The cultures were supplemented with 500 U/ml of IL-2. As shown in Figure 5, there is robust expansion (up to 100-fold) of Treg cells using the Dynabeads® CD3/CD28 approach.

For clinical research, Treg cells can be generated by expanding CD4⁺ T cells (Dyna® CD4 Negative Isolation Kit) with Dynabeads® CD3/CD28 in the presence of rapamycin for 2 weeks (Figure 6).

Treg cells in clinical applications

The Dynabeads® Regulatory CD4⁺ CD25⁺ T Cell Kit can be used to isolate ≥95% pure CD4⁺ CD25⁺ Treg cells, and more than 80% of the

isolated CD25⁺ cells express the FoxP3 transcription factor. Dynabeads® Treg Expander reagent can expand human CD4⁺ CD25⁺ Treg cells up to 100-fold during 2–3 weeks of culture while retaining their suppressive phenotype and expression of CD25 and FoxP3. Such expansion will facilitate further characterization of Treg cells as well as the evaluation of their potential in clinical applications. For more information or to place an order, visit www.invitrogen.com/bioprob56. ■

References

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Product	Application	Quantity	Cat. no.
Dynabeads® Regulatory CD4 ⁺ CD25 ⁺ T Cell Kit	Treg cell isolation	2 × 5 ml	113.63D
Dynabeads® Human Treg Expander	Treg expansion	2 ml	111.29D
Dynabeads® CD3	Short-term stimulation/suppression assays	5 ml	111.51D
Dynabeads® CD3/CD28	T cell expansion in preclinical research	10 ml	111.41D
Dynabeads® ClinExVivo™ CD3/CD28	T cell expansion in clinical research	10 ml	402.03D
Dyna® CD4 Negative Isolation Kit	CD4 ⁺ T cell isolation	5 ml	113.17D
CellTrace™ CFSE Cell Proliferation Kit	Cell proliferation kit for flow cytometry	1 kit	C34554