

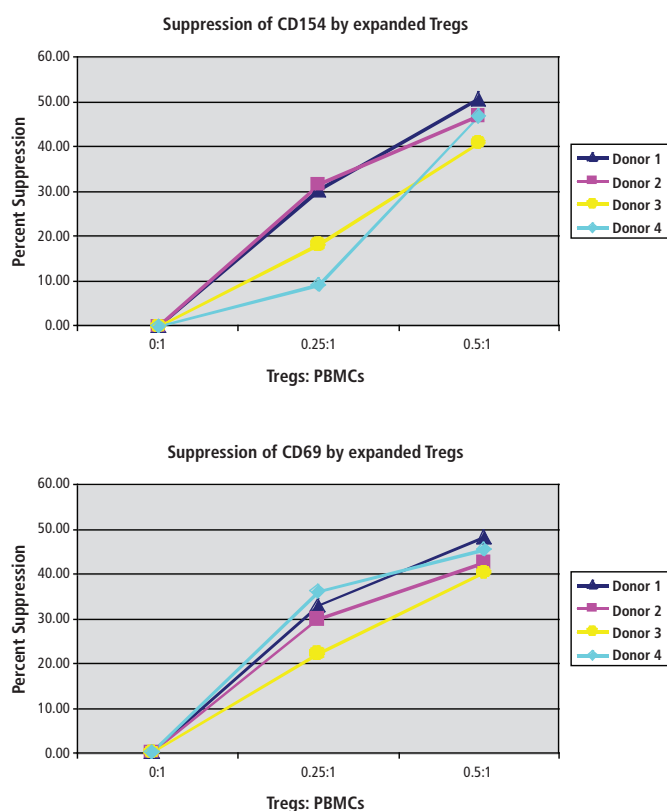
# BD FastImmune Human Regulatory T Cell Function Kit

## Features

Assess the function of expanded regulatory T cells (Tregs) in just one day

Detects the Treg-mediated suppression of the activation markers CD69 and CD154 on responding T cells using flow cytometry

Provided as a convenient cocktail of CD3, CD4, and CD25 with CD154 and CD69 as drop-in reagents



**Figure 1.** Treg-mediated suppression of CD154 (top) and CD69 (bottom) (n=4).

CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>CD45RA<sup>+</sup> sorted Tregs were expanded in culture for 14 days. At a 0.5:1 Treg:PBMC ratio, Tregs suppressed the frequency of CD4<sup>+</sup>CD154<sup>+</sup> and CD4<sup>+</sup>CD69<sup>+</sup> lymphocytes by approximately 46% and 44% (on average) respectively, when PBMCs were stimulated with anti-CD3/CD28 beads for 7 hours.

As published data on the immunosuppressive potential of Regulatory T Cells (Tregs) has accumulated, enthusiasm for their potential application has intensified. BD Biosciences has a large portfolio of products relevant to Treg research, including this BD FastImmune™ Human Regulatory T Cell Function Kit, and the portfolio continues to expand to support new discoveries.

Tregs are a rare cell population representing 5–10% of all CD4<sup>+</sup> cells, and prevent aberrant immune responses. Low numbers of Tregs, or functionally defective Tregs, can lead to autoimmune diseases such as type I diabetes and graft-vs-host disease (GvHD). Tregs suppress effector T cells by inhibiting activation, proliferation, differentiation, and effector function.<sup>1,2</sup>

The Human Regulatory T Cell Function Kit measures the expression of activation markers CD69 and CD154 on effector T cells. A decrease in the levels of activation markers in the presence of Tregs may indicate the suppressive function of Tregs.

## How the assay works

Varying concentrations of expanded Tregs are co-cultured with responding effector cells in the presence of T-cell specific stimuli such as CD3/CD28 beads. To capture transiently expressed CD154 on the cell surface, APC-labeled CD154 reagent is present during the entire stimulation time. After seven hours of activation, the frequency of CD154-positive and/or CD69-positive effector T cells is compared in the presence and absence of Tregs.

The BD FastImmune Human Regulatory T Cell Function Kit contains a 3-color cocktail of CD3 PerCP-Cy™5.5 (clone SK7), CD4 FITC (clone SK3), CD25 PE (clone 2A3), and single color CD154 APC (clone 89-76) and CD69 PE-Cy™7 (clone L78) as drop-in reagents. BD FastImmune EDTA is also included. To accommodate various flow cytometer configurations, this assay can be stained as a 4-color (by excluding CD69 PE-Cy7) or a 5-color experiment.

## Tools for the sorting of Tregs

In addition to the Treg functional assay, the BD Human Regulatory T Cell Sorting Kit is also available. The kit contains a 3-color reagent cocktail that can be used to identify and sort CD4<sup>+</sup>CD25<sup>int/high</sup>CD127<sup>low</sup> expandable natural Treg populations. The reagent cocktail includes CD4 PerCP-Cy5.5 (clone L200), CD25 PE (clone 2A3), and CD127 Alexa Fluor® 647 (clone 40131). The one-step pre-mixed cocktail simplifies Treg identification and significantly enhances enrichment of Treg populations by 2 to 4 times compared to gating on CD25<sup>high</sup> alone.

This kit also includes CD45RA FITC (clone HI100) as an optimized drop-in. Publications by Hoffmann<sup>3</sup> and Miyara<sup>4</sup> have reported that isolation of the CD45RA<sup>+</sup> subset of Tregs may provide an increased percentage of FoxP3<sup>+</sup> Tregs with greater suppressive capacity post enrichment compared to CD45<sup>-</sup> Tregs.

Visit [bdbiosciences.com/tcell](http://bdbiosciences.com/tcell) for more information.

# BD FastImmune Human Regulatory T Cell Function Kit

## References

1. Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? *Int Immunol*. 2009;21:1105-1111.
2. Askenasy N, Kamnitz A, Yarkoni S. Mechanisms of T regulatory cell function. *Autoimmun Rev*. 2008;7:370-375.
3. Hoffmann P, Eder R, Boeld TJ, et al. Only the CD45RA+ subpopulation of CD4+CD25high T cells gives rise to homogeneous regulatory T-cell lines upon in vitro expansion. *Blood*. 2006;108:4260-4267.
4. Miyara M, Yoshioka Y, Kitoh A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity*. 2009;30:899-911.

## Ordering Information

Description	Contents	Size	Cat. No.
Human Regulatory T Cell Function Kit	Human Regulatory T Cell Function Cocktail (CD3 PerCP-Cy5.5, CD4 FITC, CD25 PE), CD154 APC, CD69 PE-Cy7, and EDTA Solution	50 Tests	648956

## Related Products

Description	Contents	Size	Cat. No.
Human Regulatory T Cell Sorting Kit	Human Regulatory T Cell Sorting Cocktail (CD4 PerCP-Cy5.5, CD25 PE, CD127 Alexa Fluor® 647), CD45RA FITC, and compensation controls	10 Tests 1 Test = 1 x 10 <sup>6</sup> cells	560753



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