iba Solutions For Life Sciences

Cell Selection & Expansion

LABEL-FREE REGULATORY T CELLS

Isolation and staining with CD4, CD25 and CD45RA Fab Streptamers®

INTRODUCTION

Regulatory T cells (Treg) classically characterized as $CD4^+CD25^{high}$ expressing cells[1-7] are an indispensable part of the immune system for maintaining dominant self-tolerance and immune homeostasis. Their central role in the control of immune responses is mediated by their ability to suppress activation, proliferation and effector function of a variety of immune cells like $CD4^+$, $CD8^+$, NK and NKT cells, as well as B-cells and antigen presenting cells. The importance of Treg cells in the prevention of autoimmune disease, immunopathology and allergy, as well as in the retention of allograf tolerance is well described[8].

Due to their importance in immune regulation the therapeutic potential of adoptively transferred Treg cells resembles a major target for clinical applications[9]. Several preclinical and clinical data suggest beneficial effects from Treg infusional therapy in various autoimmune diseases like graft-versus-host disease (GvHD)[10], graft rejection, Type1 diabetes (T1D)[11-13], colitis[14,15] and several forms of allergy[16].

But still, translating Treg cell therapy into the clinic bears diverse technical challenges mainly related to complicated isolation procedures necessary to select Tregs after *in vitro* expansion or directly from *in vivo* sources[14]. Due to the lack of a unique Treg specific surface marker, positive selection requires a combination of at least two markers to obtain highly enriched Tregs circumventing expensive and laborious negative selection strategies. In addition, recent studies clearly indicated that Treg cells do not represent a homogenous subpopulation but can rather be subdivided into phenotypically and functionally different subsets based on differential cell surface marker expression. One of the most extensively studied markers is CD45RA, which characterizes regulatory T cells with a preserved superior suppressive capacity even after expansion and the ability of continued proliferation both *in vitro* and *in vivo*[17,18]. These findings suggest that isolation/expansion of $CD45RA^+CD4^+CD25^{high}$ T cells is the best strategy for adoptive Treg-cell therapies[17].

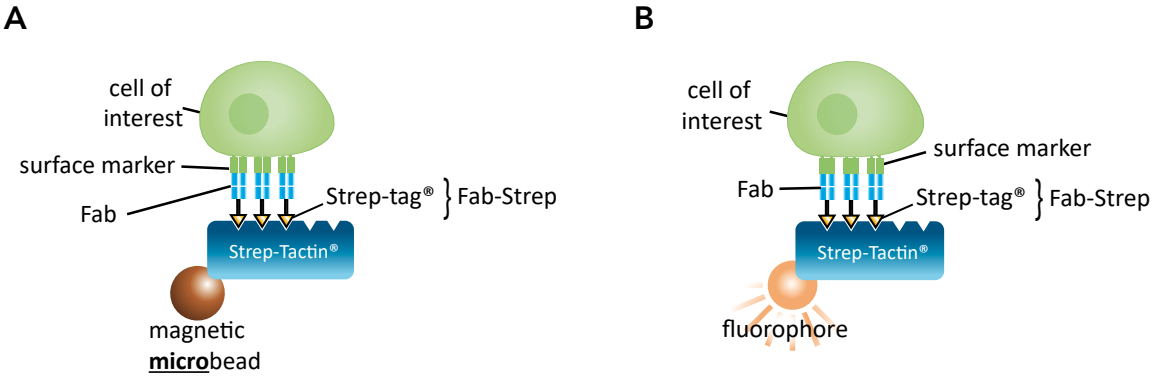

Figure 1. Fab Streptamer® complexes for reversible cell isolation (A) or for reversible staining (B).

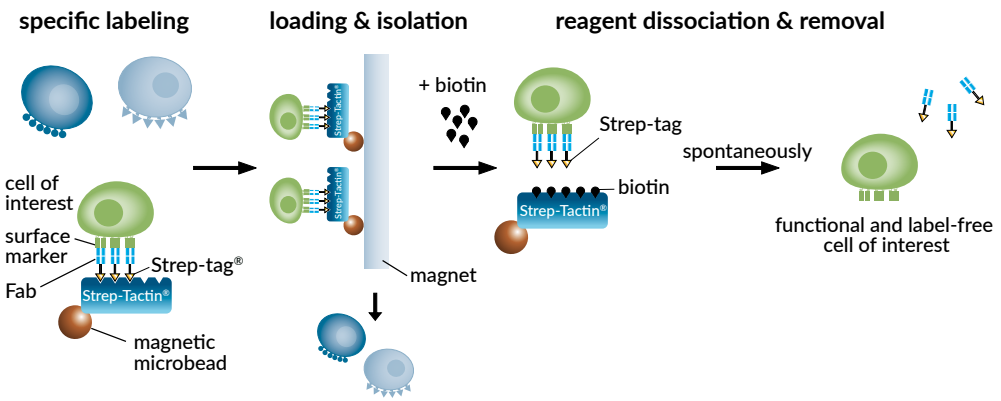

Figure 2. Cell isolation with reversible Fab Streptamer® reagents.
Low-affinity Fab-Streps are reversibly multimerized on Strep-Tactin® microbeads forming a Fab Streptamer® for cell isolation. Treatment of isolated cells with the competing Strep-Tactin® ligand biotin causes disruption of the Fab Streptamer® complex and results in spontaneous dissociation of all monomeric Fab-Streps from the target cell surface.

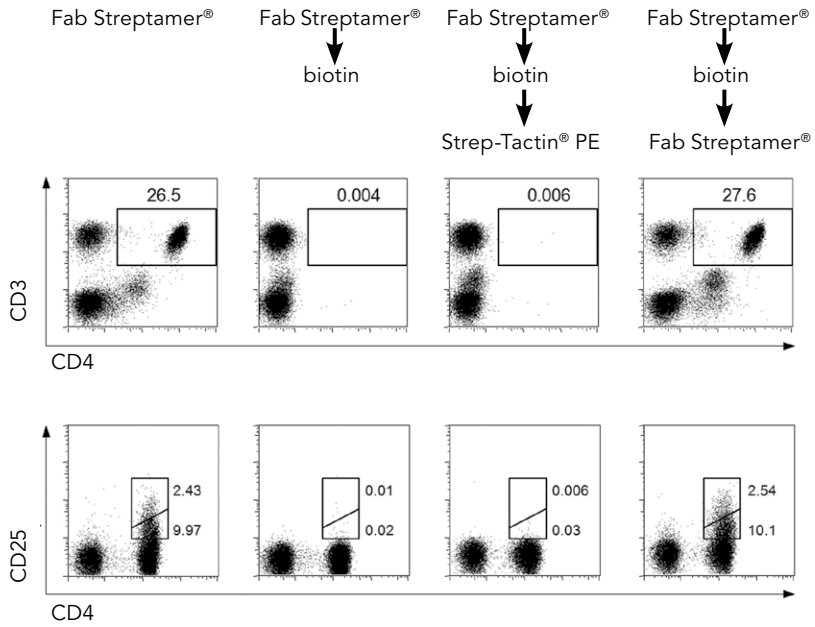

Figure 3. Reversible staining with fluorescent CD4 and CD25 Fab Streptamers®.
CD4 or CD25 fluorescent Fab Streptamers® were used to stain CD4⁺ (top) or CD25⁺ cells (bottom), respectively. Cells were analyzed either before or after treatment with biotin. Remaining Fab-Streps could not be detected after subsequent wash steps using PE-labeled Strep-Tactin®. Secondary Fab Streptamer® staining of reversibly stained cells served as control.

RESULTS and DISCUSSION

During multiparameter positive selection strategies, residing cell bound selection markers pose a general problem like e.g. antibody mediated receptor blockade (like the IL-2R chain [CD25]). In addition, strict rules appear for cell isolation under GMP conditions and especially for the infusion of substantial amounts of irreversibly surface bound selection markers into the patient.

The Streptamer® technology now circumvents these severe problems, as target cells can be entirely liberated from all components of the Streptamer® reagents. After cell isolation with magnetic Fab Streptamers® (Fig. 1A) or after cell staining with fluorescent Fab Streptamers® (Fig. 1B), Fab Streptamers® can be efficiently removed from the labeled cells by gentle biotin (vitamin H) mediated dissociation of the Fab-Strep – cell complex from Strep-Tactin® magnetic beads (Fig. 2) or from Strep-Tactin® PE, respectively. Subsequent liberation of the cells from all single low affinity Fab-Streps is then achieved by conventional wash steps, and no remaining Fab-Streps can be detected on the surface of previously Fab Streptamer® stained cells (Fig. 3). In addition, the combined use of

reversible Fab Streptamers® CD4, CD25 and CD45RA now allows for the first time to positively isolate Tregs over multiple markers by serial magnetic enrichment without any preceding negative selection steps (Fig. 4). $CD4^+CD25^+$ Treg cells can be highly purified by a double magnetic selection from PBMCs in a two-step approach. First, $CD4^+$ cells are positively selected by CD4 magnetic Fab Streptamers® and entirely liberated from the reagents. After the second isolation step using CD25 magnetic Fab Streptamers®, the $CD4^+CD25^+$ double positive target population can be obtained. Final selection for CD45RA then allows enrichment of a triple positive highly specific Treg cell preparation (Fig. 4). The complete removal of all surface bound selection reagents after purification not only assures the reinfusion of minimally manipulated cells but also eliminates potential clinical side effects of remaining selection reagents.

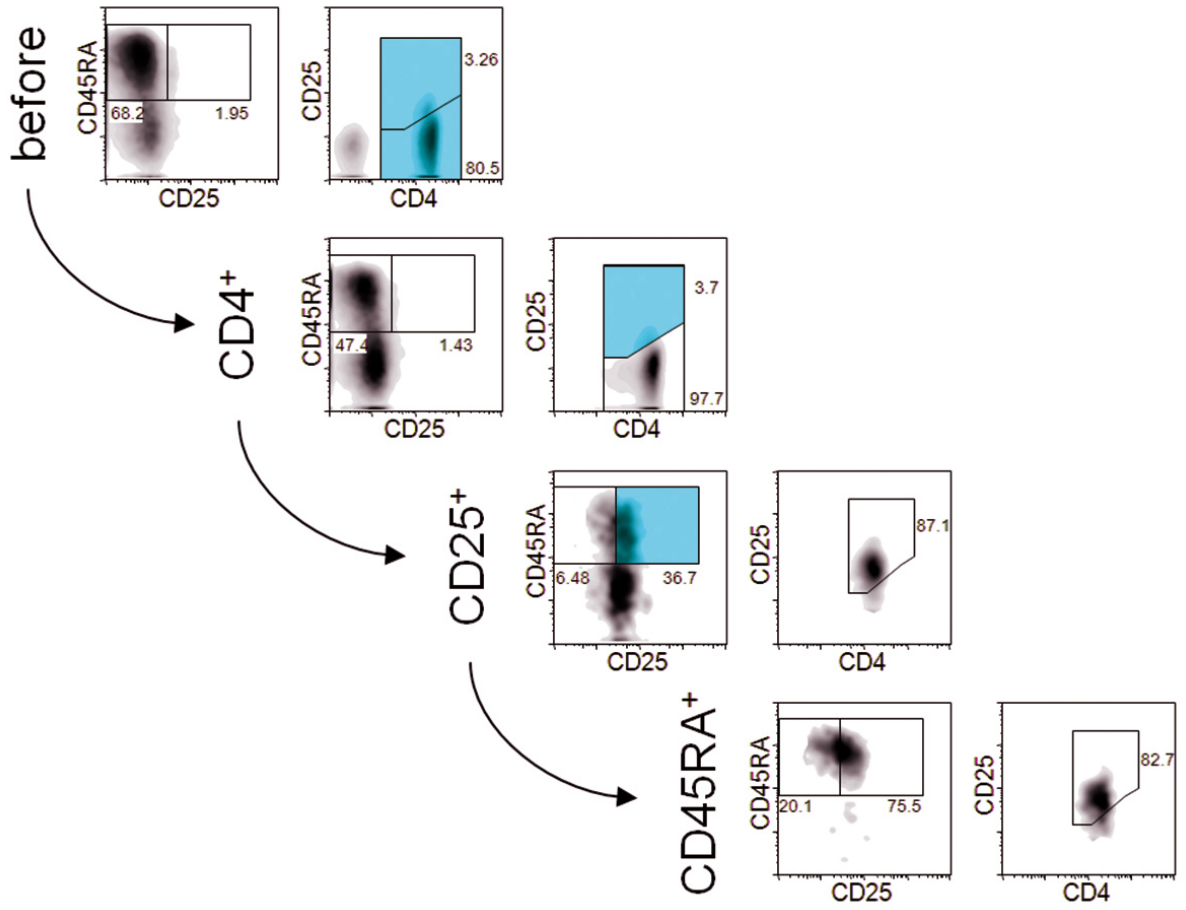

Figure 4. Serial magnetic enrichment of $CD4^+CD25^+CD45RA^+$ T cells.
Cells were incubated with CD4 magnetic Fab Streptamers® (Figure 2a) for preselection of $CD4^+$ cells. The resulting positive fraction was then further processed by biotin treatment and subsequent washing to remove all CD4 selection reagents. In two subsequent serial positive enrichment steps for CD25 and CD45RA, a triple positive Treg target population was selected.

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Version AN32-02-0002