iba Solutions For Life Sciences

Cell Selection & Expansion

LABEL-FREE CENTRAL MEMORY T CELLS

Isolation and staining with CD8α and CD62L Fab Streptamers®

INTRODUCTION

T cells play a central role in cell-mediated immunity. The development of cytotoxic T lymphocyte (CTL) responses is necessary for the control of a variety of bacterial and viral infections as well as certain types of malignancies. CTLs, which are largely $CD8^+$, traffic to peripheral sites of infection and specifically eliminate their target cells[1].

In the recent years the therapeutic capacity of these cells has opened up new avenues especially for the development of (personalized) cellular therapies based either on highly enriched primary T cell preparations or purified from *in vitro* cultures. These therapies cover a broad spectrum of applications in order to restore e.g. antiviral immunity or improving strategies to treat certain types of cancer[2-6].

In order to maintain protection, long-living memory T cells are generated that persist throughout an individual's lifespan[1] without the need to re-encounter antigen eventually. With the identification of functionally and phenotypically distinct subsets of memory T cells – so called central (T_{CM}) and effector memory (T_{EM}) T cells – however a division of labor between the different $CD8^+$ T cell types has become evident[7,8].

Especially $CD8^+CD45RA^-$ T_{CM} that are characterized by high expression of the lymph node homing molecule CD62L, have been shown to retain phenotypic as well as functional properties of memory T cells upon adoptive transfer[9-11] and were able to confer protection. Notably this was also observed after transfer of *in vitro* expanded T cell clones that were initially derived from $CD62L^+$ central memory T cells[10]. Taken together, due to their long-term *in vivo* persistence as well as their conserved functional properties, $CD62L^+CD8^+CD45RA^-$ central memory T cells might be superior for adoptive T cell therapy[12].

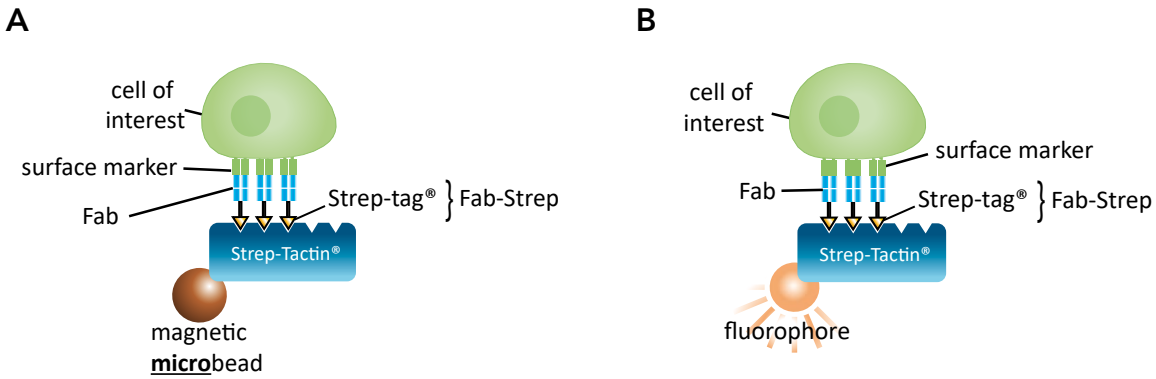

Figure 1. Fab Streptamer® complexes for reversible cell isolation (A) or for reversible staining (B).

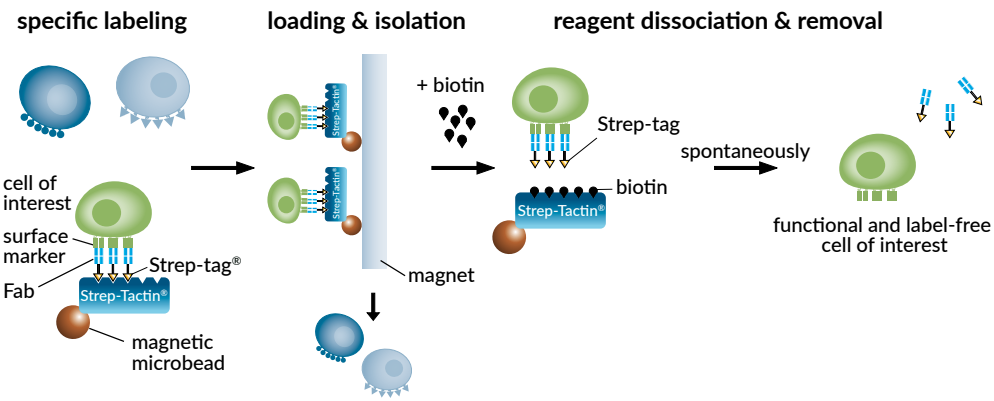

Figure 2. Cell isolation with reversible Fab Streptamer® reagents.
Low-affinity Fab-Streps are reversibly multimerized on Strep-Tactin® microbeads forming a Fab Streptamer® for cell isolation. Treatment of isolated cells with the competing Strep-Tactin® ligand biotin causes disruption of the Fab Streptamer®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

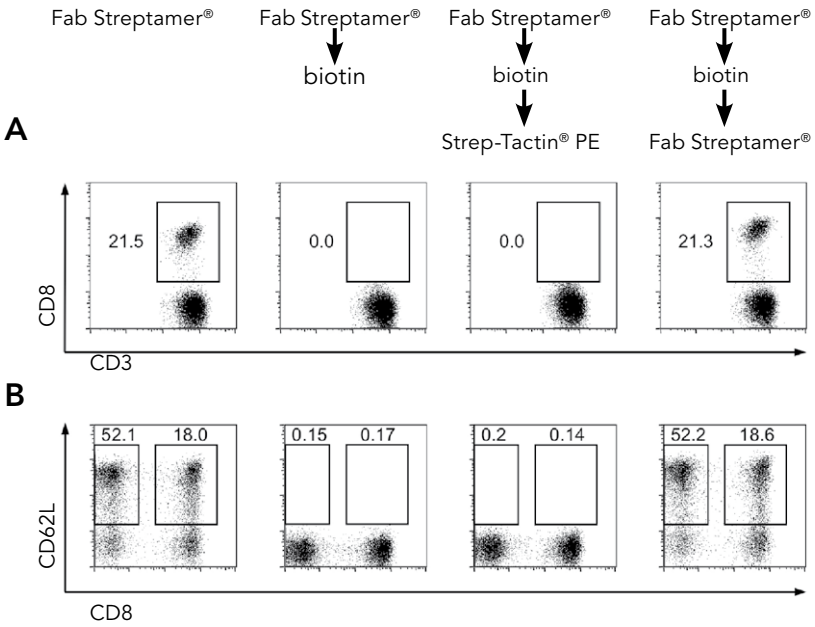

Figure 3. Reversible staining with fluorescent CD8 and CD62L Fab Streptamers®.
CD8 or CD62L fluorescent Fab Streptamers® were used to stain CD8⁺ (A) or CD62L⁺ cells (B), respectively. Cells were analyzed either before or after treatment with biotin. Remaining Fab-Streps could not be detected after subsequent washing steps using (uncomplexed) PE-labeled Strep-Tactin®. Secondary Fab Streptamer® staining of reversibly stained cells served as control. CD8 and CD62L expression of live CD3⁺ T cells is shown. Numbers in dot plots indicate percent of cells within gates.

RESULTS and DISCUSSION

One difficulty in selecting and isolating the desired target cells is that remaining selection reagents often have a negative impact on the cell product (e.g. by reagent-mediated receptor blockade) often completely eliminating its pivotal function. 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 stained cells (Fig. 3). In addition, the combined use of reversible Fab Streptamers® directed against CD8 and CD62L now allows for the first time to positively isolate T_{CM}s by serial positive magnetic enrichment (Fig. 4). An additional CD45RA depletion of the obtained cell product can also be

used to further discriminate between naïve and memory (CD45RA$^-$) T cells. CD62L$^+$ T cells were highly purified by a double magnetic selection from fresh PBMCs in a two-step approach (Fig. 4). First, CD8$^+$ cells were positively selected by magnetic CD8 Fab Streptamers® and entirely liberated from the reagents. After the second isolation step with magnetic CD62L Fab Streptamers®, the CD62L$^+$CD8$^+$ T cell target population was obtained as a highly pure population. Serial magnetic enrichments can also be performed for other selection strategies. For instance antigen-specific CD8$^+$ T cells can be selected by the combined use of CD8 Fab Streptamers® followed by an antigen-specific selection with MHC I Streptamers®.

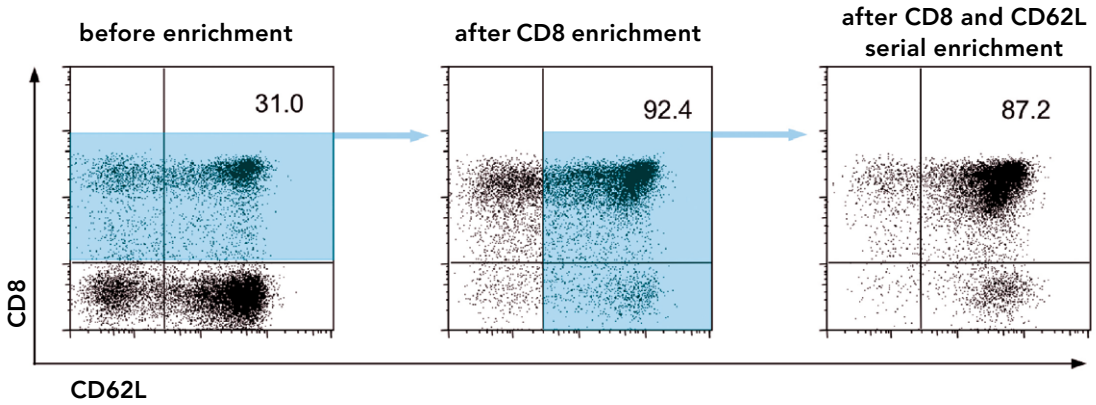

Figure 4. Serial magnetic enrichment of CD8$^+$CD62L$^+$ T cells
Cells were incubated with CD8 magnetic Fab Streptamers® for preselection of CD8$^+$ cells. The resulting positive fraction was then further processed by biotin treatment and subsequent washing to remove all CD8 selection reagents. In a second step, the target CD8$^+$CD62L$^+$ T cell population was then highly enriched from the pre-selected CD8$^+$ cell pool with CD62L magnetic Fab Streptamers®. Live lymphocytes for the respective positive and negative fractions of both selection steps are shown.

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