iba Solutions For Life Sciences

Cell Selection & Expansion

# LABEL-FREE T-LYMPHOCYTES

## Isolation with CD3 Fab Streptamers®

### INTRODUCTION

T cells play a central role in cell-mediated immunity. These cells divide into several subsets (e.g. $CD4^+$ and $CD8^+$ T cells), which not only control different adaptive immune responses but also mediate effector function necessary for protection against pathogens and certain types of malignancies. In the recent years the therapeutic capacity of these cells has opened up new avenues for the development of (personalized) cellular therapies based especially on highly enriched primary T cell preparations. These therapies cover a broad spectrum of applications ranging from the restoration of antiviral immunity or strategies to treat certain types of cancer but also to the induction of selective immune suppression[1-6].

T cell recognition is mediated by clonotypically distributed αβ or γδ T cell receptors (TCRs) that specifically interact with the peptide loaded MHC-complexes of antigen-presenting cells. The antigen-specific chains of the TCR do not possess signaling domains but instead utilize the CD3 signaling complex consisting of CD3δε, CD3γε heterodimers and the homodimeric ζζ signaling chains[7]. Binding of the TCR/CD3 complex initiates a cascade of signaling that starts with the activation of several cytoplasmic protein tyrosine kinases. Recruitment of the CD4 (or CD8) co-receptor and its associated tyrosine kinase, Lck, into the vicinity of the TCR complex is believed to induce phosphorylation of CD3 proteins, which ultimately leads to downstream signal progression[8].

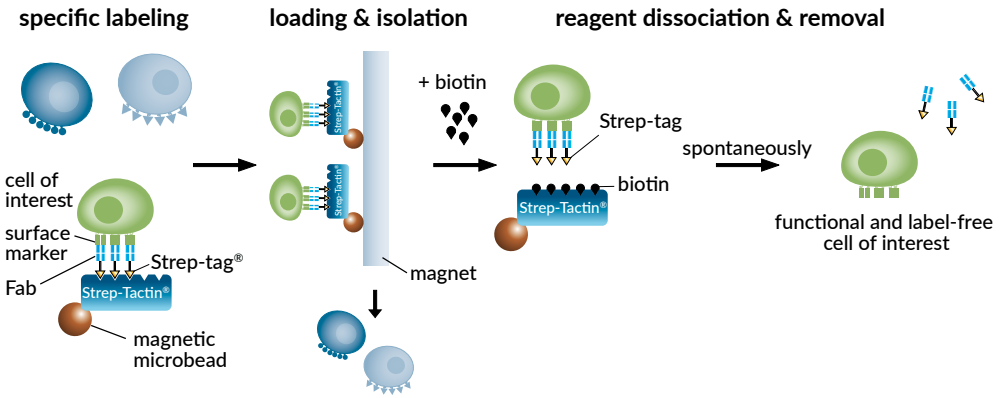


**Figure 1. Cell isolation with reversible Fab Streptamer® reagents.**
Low-affinity Fab-Streps are reversibly multimerized on Strep-Tactin® microbeads forming a Fab Streptamer® for cell isolation. Treatment of isolated cells with the competing Strep-Tactin® ligand biotin causes disruption of the Fab Streptamer® complex and results in spontaneous dissociation of all monomeric Fab-Streps from the target cell surface.

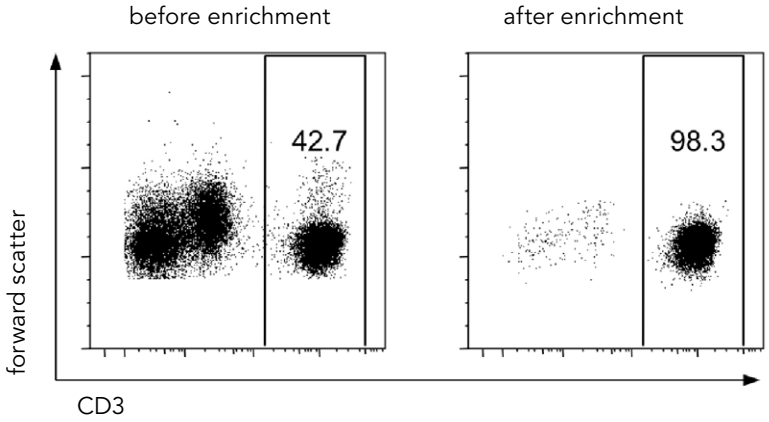


**Figure 2. Magnetic enrichment of CD3⁺ T cells from PBMCs.**
For isolation, PBMCs were incubated with CD3 Fab Streptamers®. During the isolation the cells were further processed by biotin treatment and subsequent washing to remove all CD3 selection reagents. Dot plots show cells before (left) and after enrichment (right).

## RESULTS and DISCUSSION

Due to the essential role in T cell biology, targeting CD3 excerpts profound influence on labeled cells and is widely used to either stimulate- or inhibit T cell responses[9-11]. Remaining isolation reagents (like e.g. anti-CD3 mAbs) may influence transferred T cells in terms of early signal transduction, cellular activation and proliferation. Therefore, complete removal of reagents becomes crucial for obtaining unaltered cells. By using the Streptamer® technology target cells can be entirely liberated from all selection reagents (Fig. 1). After magnetic cell isolation, for instance the isolation of CD3 T cells (Fig. 2), the Fab Streptamers® can be easily removed from the target cells by a gentle biotin (vitamin H) mediated dissociation of the Fab-Strep:Strep-Tactin® complex. Subsequent removal of the detached reagents including low affinity Fab-Streps and biotin is then achieved by wash steps.

## REFERENCES

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