Cell Selection & Expansion

Application Note

DEPLETION OF CELL POPULATIONS FROM DIFFERENT SAMPLES

An affinity chromatography approach

Introduction

The specific depletion of cell populations from donor material can be crucial for different experimental approaches. For example, it can help to unravel the effects that a single cell population has on a whole system, to increase safety by eliminating unwanted cells or to get rid of cell populations and subpopulations that potentially impair the experimental outcome. In addition, cell depletion might be necessary if an initial positive selection approach yielded a high amount of non-target cells resulting in a low purity of the desired population.

Here we introduce different options to utilize Strep-Tactin® TACS Agarose columns for highly efficient cell depletion. Strep-Tactin® TACS Agarose was developed for Traceless Affinity Cell Selection based on our Strep-tag® technology. Strep-Tactin® is a streptavidin derivative that has optimized binding properties to the Twin-Strep-tag® (affinity: nM range), but still binds strongly to biotin (affinity: 100 pM –100 fM). This allows immobilization of, for example, biotinylated **(Fig. 1A)** or Twin-Strep-tagged **(Fig. 1B)** antibodies against specific cell surface markers inside the columns, making the columns potentially suitable for cell depletion approaches.

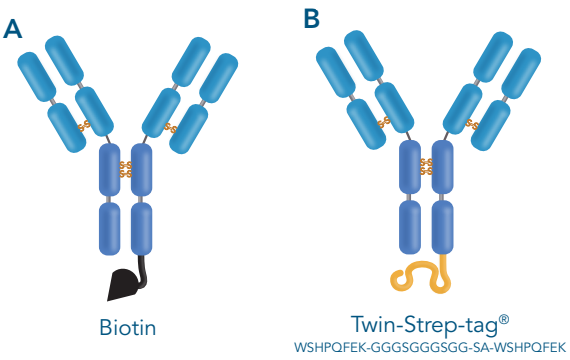

Fig 1. Monoclonal antibodies for Strep-Tactin® TACS Agarose based enrichment tagged with Biotin (A) or Twin-Strep-tag® (B). Both tags can be used for binding on the agarose.

The starting materials that we tested to evaluate successful removal of different cell populations from a sample include peripheral blood mononuclear cells (PBMCs), buffy coat and pre-enriched monocytes. We mainly used biotinylated antibodies, although antibodies fused to a Twin-Strep-tag® are also an option. Specific cell populations were efficiently depleted from all sample types with both types of tagged antibodies in only 10 minutes.

Methods

Cell depletion from PBMCs

1 x 10^7 PBMCs in 100 µl buffer for cell isolation (Buffer CI: PBS containing 0.5% BSA and 1 mM EDTA) were pre-incubated with 0.5 µg biotinylated antibody against surface markers CD235a (erythrocytes), CD14 (monocytes), CD3 (T cells), CD15 (granulocytes), CD19 (B cells) or CD36 (thrombocytes) for 5 min at room temperature. For comparing T cell depletion using biotinylated antibodies with depletion using Twin-strep-tagged antibodies, PBMCs were incubated with 1 µg antibody, respectively. Samples were loaded onto Strep-Tactin® TACS Agarose columns containing 1 ml bed volume (maximal binding capacity: 1 x 10^8 cells). Directly after samples entered the bed completely, 10 ml Buffer CI was applied twice to elute non-bound cells. Cell depletion was evaluated by flow cytometry.

Cell depletion from buffy coat

500 µl buffy coat were pre-incubated with 5 µg biotinylated antibody against CD14 (monocytes) for 5 min at room temperature. The samples were loaded onto Strep-Tactin® TACS Agarose columns containing 1 ml bed volume. Directly after samples entered the bed completely, 3 ml Buffer CI was applied to elute non-bound cells. Monocyte depletion was evaluated by flow cytometry.

Cell depletion from pre-enriched monocytes

Monocytes were pre-enriched from 5 ml undiluted buffy coat using IBA's Fab-TACS® technology for label-free cell isolation. 2.5 x 10^6 monocytes in 200 µl Buffer CI were incubated with 0.5 µg anti-CD235a and anti-CD15 for 5 min at room temperature to remove remaining erythrocytes and granulocytes. The sample was loaded onto Strep-Tactin® TACS Agarose columns. Directly after samples entered the bed completely, 10 ml Buffer CI was applied twice to elute non-bound cells. Monocyte purity was determined by flow cytometry.

All commercially available reagents used for the depletion are listed in the Appendix (Table 1).

Results and discussion

Efficient cell depletion from PBMCs

The removal of specific cell populations from samples is required for some experimental approaches. We tested whether efficient depletion of different cells from PBMCs can be achieved using Strep-Tactin® TACS Agarose columns in combination with biotinylated antibodies. Nearly all targeted cell populations including T cells, B cells, erythrocytes, monocytes and platelets could be depleted with on average ≥ 99% efficiency **(Fig. 2A-E).** For granulocytes, this efficiency was a bit lower (98.5%, **Fig. 2F**), which can be explained by a high variability in starting cell numbers. Combining different antibodies can serve as a negative cell selection approach to enrich a specific cell type of interest **(see Application Note: Negative Cell Selection Using Affinity Chromatography)**.

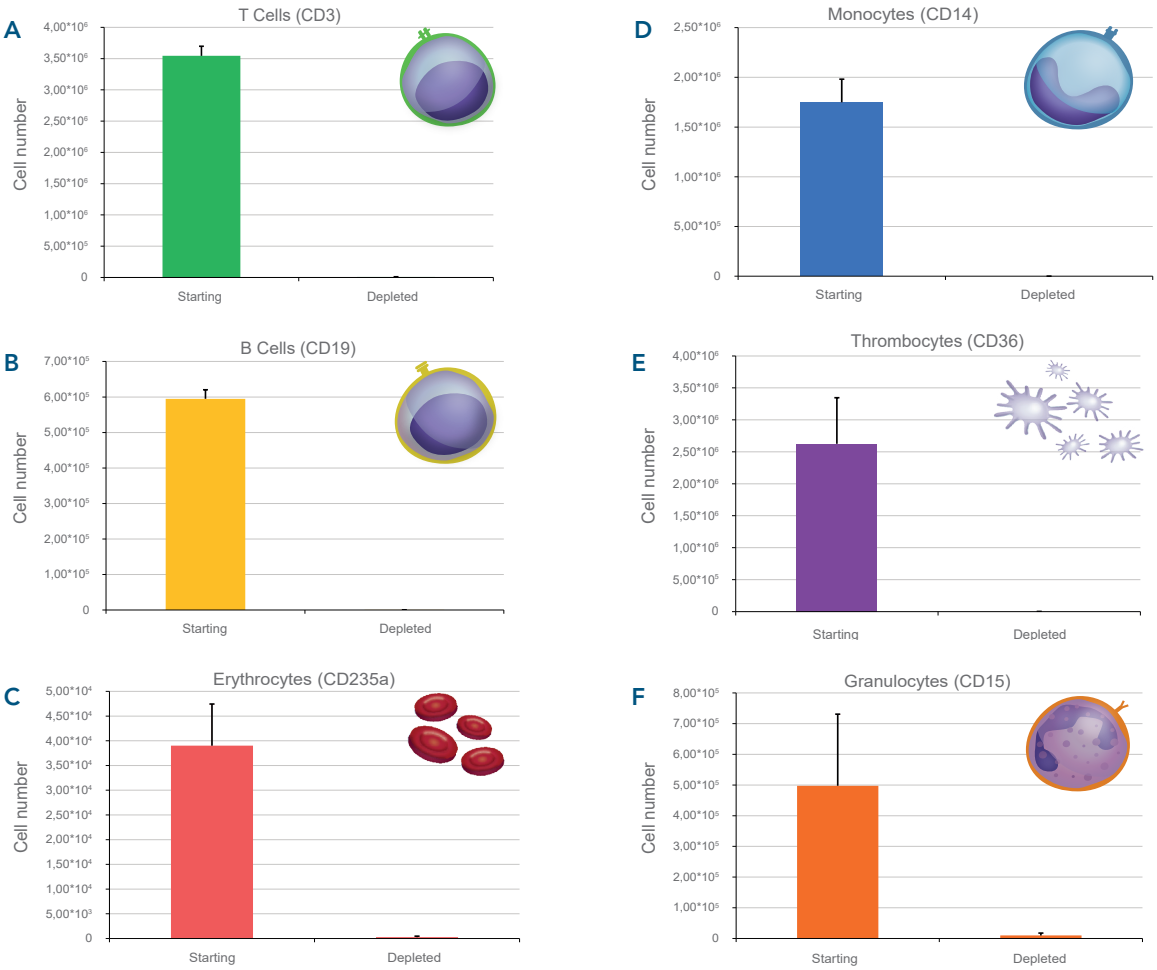

Fig. 2 Depletion of target populations from PBMCs using biotinylated monoclonal antibodies and Strep-Tactin® TACS Agarose columns (n = 3). Depletion efficiency was determined by flow cytometry.

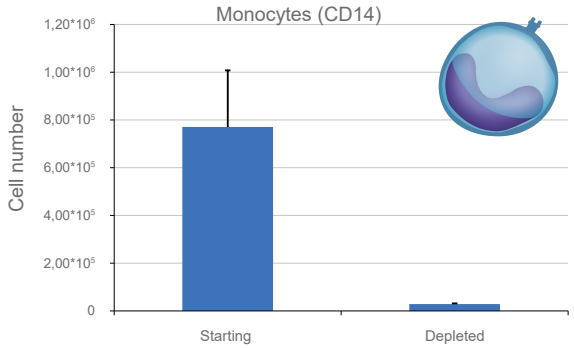

Fig. 3 Direct depletion of monocytes from buffy coat using biotinylated monoclonal antibodies and Strep-Tactin® TACS Agarose columns (n=2). Depletion efficiency was determined by flow cytometry.

Efficient cell depletion from buffy coat

The preparation of PBMCs from whole blood or buffy coat is a time-consuming procedure and can lead to cell loss. Therefore, we tested whether cell depletion is also possible directly from undiluted buffy coat. Indeed, monocytes could be depleted with a mean efficiency of 95.7% **(Fig. 3)**, showing that pre-isolation of PBMCs is not necessary for cell depletion using Strep-Tactin® TACS Agarose columns in combination with biotinylated antibodies.

Efficient cell depletion from pre-enriched monocytes

The depletion of unwanted cell populations can also serve as an additional step after a positive selection procedure to get an ultra-pure cell preparation.
We enriched monocytes from buffy coat, which resulted into an increase in purity from 0.1% **(Fig. 4A)** to 82.3% **(Fig. 4B)**. Erythrocytes and granulocytes were the main non-specific cell populations after monocyte enrichment and therefore we performed an additional depletion step for both cell types. This increased monocyte purity to 95.2% **(Fig. 4C)**, showing that an additional depletion step using Strep-Tactin® TACS Agarose columns is a fast and efficient way to increase the quality of a sample.

Efficient cell depletion with Twin-strep-tagged antibodies

Biotin binds to Strep-Tactin® with a high affinity. We tested whether antibodies that have a Twin-Strep-tag® are also an option for cell depletion. Using a Twin-strep-tagged antibody against CD3 depleted 99.7% of targeted cells, which was very similar to using biotinylated antibodies (99.8%, **Fig. 5**). Although biotinylated antibodies have a higher affinity to Strep-Tactin® compared to the Twin-Strep-tag®, cell depletion was equally efficient. The advantages of Twin-Strep-tag® monoclonal antibodies are the easier way of production and purification as well as the possibility to elute the depleted cells by adding biotin. Furthermore, the position of the Twin-Strep-tag® on the antibody is fixed, whereas the amount and location of biotinylation can vary. In contrast, the advantage of biotinylated antibodies is that they are inert to biotin contamination after pre-purification of cells with the Fab-TACS® technology. However, successful cell depletion can be achieved with both types of antibodies.

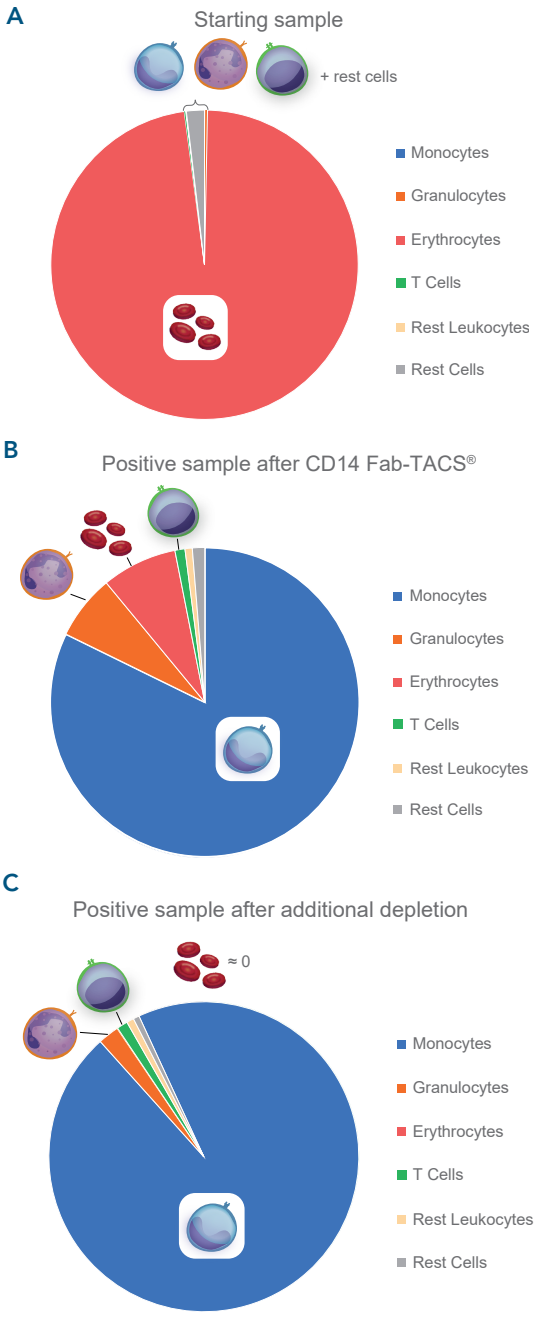

Fig. 4 Monocytes were pre-enriched from buffy coat using Fab-TACS® Technology. Purity was determined by flow cytometry before (A) and after monocyte enrichment (B) as well as after granulocyte and erythrocyte depletion (C).

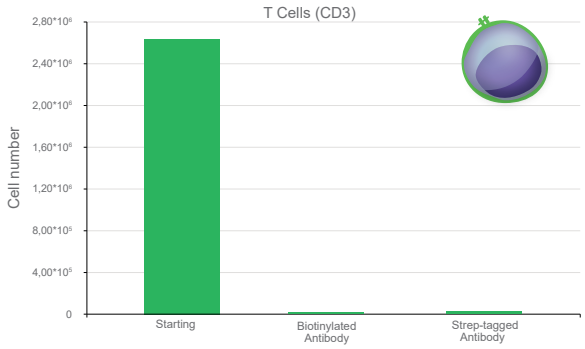

Fig. 5 Depletion of T cells from PBMCs using biotinylated or Twin-strep-tagged monoclonal antibodies and Strep-Tactin® TACS Agarose columns. Depletion efficiency was determined by flow cytometry.

Conclusion

Strep-Tactin® TACS Agarose columns combined with biotinylated antibodies can be utilized to efficiently deplete different cell populations from a variety of starting materials. The possibility to choose between biotinylated and Twin-strep-tagged antibodies contributes to a great flexibility and broad applicability of this method. With only 10 minutes hands-on time this approach is one of the fastest depletion methods currently available on the market.

Appendix

Name	Manufacturer	Catalog Number
Strep-Tactin® TACS Agarose Column, 1 ml	IBA Lifesciences	6-6310-001
10x Buffer CI, 85 ml	IBA Lifesciences	6-6320-085
Anti-hu CD235a Biotin mAb	Invitrogen	13-9987-82
Anti-hu CD14 Biotin mAb	Invitrogen	13-0149-82
Anti-hu CD3 Biotin mAb	Invitrogen	13-0037-82
Anti-hu CD15 Biotin mAb	Invitrogen	13-1059-82
Anti-hu CD19 Biotin mAb	Invitrogen	13-0199-82
Anti-hu CD36 Biotin mAb	BioLegend	336218

Table 1 Commercially available reagents used for the depletion

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