Cell Selection & Expansion

Application Note

NEGATIVE CELL SELECTION USING AFFINITY CHROMATOGRAPHY

A fast and simple method

Introduction

Different isolation approaches aim at minimally affecting cells to maintain their original phenotype. In comparison to positive selection, in which cells of interest are directly labeled and isolated, negative selection describes a method in which all unwanted cells are labeled and depleted. The advantage of using negative selection is that cells of interest remain completely "untouched" during the isolation procedure. Avoiding the direct binding of e.g. surface marker-specific antibodies reduces activation and thereby keeps the cells in a more natural state. However, to efficiently deplete unwanted populations from a sample, the cell composition has to be known to include all relevant targets in the required antibody cocktail.

Advantages	Disadvantages
Untouched cells	Complicated to design for unknown samples
Rapid isolation	Lower purity

Table 1 Advantages and disadvantages of negative cell selection.

Here we demonstrate that our Strep-Tactin® TACS Agarose columns that were developed for positive cell isolation approaches (Traceless Affinity Cell Selection), are also applicable for negative cell selection. Strep-Tactin® is a streptavidin derivative that binds to biotin with an affinity of 100 pM – 100 fM. This allows immobilization of biotinylated antibodies against specific cell surface markers inside the columns, a property that can be employed for negative cell selection. To evaluate the efficiency of Strep-Tactin® TACS Agarose columns in this approach, we enriched $CD3^-$ $CD56^+$ natural killer (NK) cells from peripheral blood

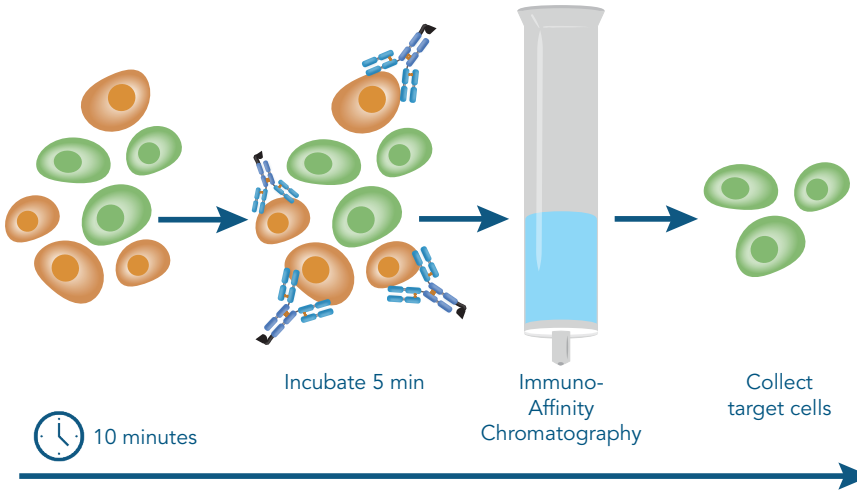

Fig. 1 Negative cell selection in 10 minutes: PBMCs are incubated with a mixture of biotinylated antibodies that bind to unwanted cells. Target cells are directly collected after passing the sample through the column.

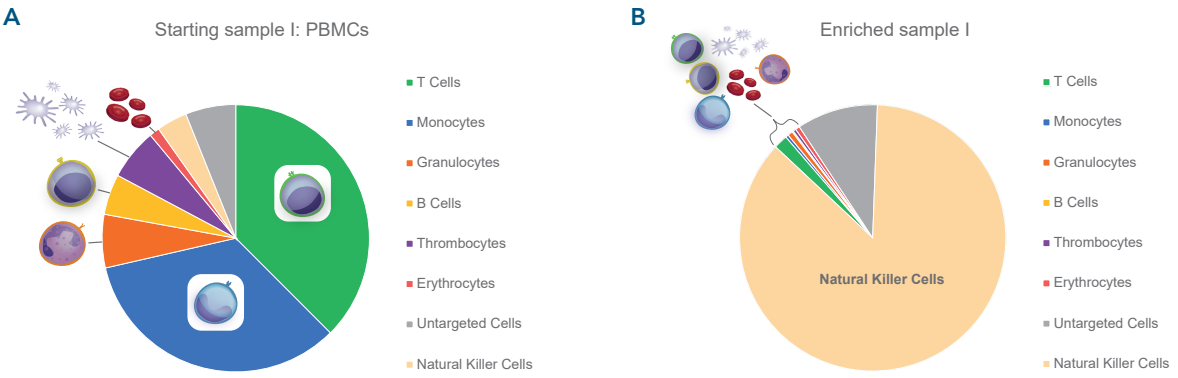

Fig. 2 Enrichment of CD56⁺ NK Cells from PBMCs by negative cell selection. The purity increased from 6% (A) to 86% (B) using Strep-Tactin® TACS Agarose columns in combination with biotinylated antibodies (one representative experiments of n = 2).

mononuclear cells (PBMCs). Combining biotinylated antibodies with our Strep-Tactin® TACS Agarose columns facilitated negative cell selection in only 10 minutes **(Fig. 1).**

Methods

For $CD3^{-}CD56^{+}$ NK negative cell selection, 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 a biotinylated antibody cocktail for 5 min at room temperature. The antibody cocktail contained 0.5 µg of each of the following antibodies: CD235a (erythrocytes), CD14 (monocytes), CD3 (T cells), CD15 (granulocytes), CD19 (B cells), CD36 (thrombocytes), CD123 (hematopoietic stem cells) and CD279 (PD-1). 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. NK cell enrichment was evaluated by flow cytometry.

All commercially available reagents used for negative cell selection are listed in the Appendix (Table 2).

Results and discussion

During a negative selection procedure, unwanted cells are removed to get a high purity of the desired cell population. We performed two independent experiments to test whether negative cell selection is possible with our Strep-Tactin® TACS Agarose columns. A representative example is shown in **Fig. 2**. NK cells were enriched from 6% **(Fig. 2A)** in the starting sample to 86% in the positive sample **(Fig. 2B)**. This experiment also highlights the difficulty of negative selection setups as the remaining non-NK cells are mostly untargeted cells. All targeted cell populations are reduced almost completely. Nevertheless, the data demonstrate that Strep-Tactin® TACS Agarose columns can be used to purify target cells via negative selection and is only limited by the antibody cocktail used.

Although we used biotinylated monoclonal antibodies **(Fig. 3A)** for negatively selecting NK cells, it is also possible, despite their lower affinity, to use antibodies that have a Twin-Strep-tag® **(Fig. 3B)**.

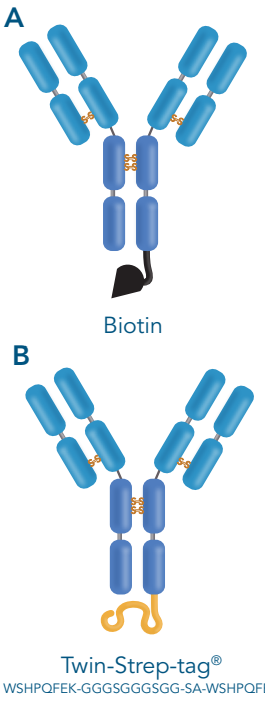

Fig. 3 Monoclonal antibodies for Strep-Tactin® TACS Agarose based enrichment tagged with Biotin (A) or Twin-Strep-tag® (B). Both tags can be used for binding on the agarose.

The advantages of Twin-Strep-tagged monoclonal antibodies are the easier way of production and purification as well as the possibility to elute the depleted cells by adding biotin. In addition, the position of the Twin-Strep-tag® on the antibody is fixed, whereas the amount and location of biotinylation can vary. In contrast, an advantage of biotinylated antibodies is the higher affinity to Strep-Tactin® compared to the Twin-Strep-tag®, which could make the negative selection process slightly more efficient. Nevertheless, negative cell selection works with both types of antibodies.

Conclusion

Strep-Tactin® TACS Agarose columns, combined with biotinylated antibodies, are applicable for negative cell selection. The possibility to choose between biotinylated and strep-tagged antibodies contributes to a great flexibility for selecting the ideal antibody cocktail for negatively selecting specific cell types. With only 10 minutes hands-on time, this approach offers a quick and well-working alternative for currently available negative cell selection methods 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 CD4 Biotin mAb	Invitrogen	13-0049-82
Anti-hu CD15 Biotin mAb	Invitrogen	13-1059-82
Anti-hu CD123 Biotin mAb	Invitrogen	13-1239-82
Anti-hu CD19 Biotin mAb	Invitrogen	13-0199-82
Anti-hu CD279 PD-1 Biotin mAb	BioLegend	329934
Anti-hu CD36 Biotin mAb	BioLegend	336218

Table 2 Commercially available reagents used for negative cell selection.

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Version AN32-06-0001