Cell Selection & Expansion

Application Note

FLEXIBLE UP- AND DOWNSCALING OF CELL ISOLATION

Efficient cell purification via affinity chromatography

Introduction

Agarose or, more specifically, agarose beads are commonly used as solid-phase support in protein purification approaches. The beads can be functionalized to allow specific binding of target proteins to the beads to purify them from a mixed sample. For the Strep-tag® technology, the agarose is coated with Strep-Tactin®, an engineered streptavidin. Strep-Tactin® binds the Strep-tag®II and even more efficiently the Twin-Strep-tag®, permitting immobilization of proteins that are fused to one of these short peptide sequences. The addition of biotin or desthiobiotin causes the elution of the captured proteins due to the higher affinity of those components to Strep-Tactin®.
The same principle is applicable to cell isolation. In this case, a strep-tagged protein is loaded onto the beads, which in turn captures a specific target cell from a sample. However, this approach is only successful with a modified agarose. We developed a Strep-Tactin® TACS Agarose that fulfills all requirements for efficient cell purification via affinity chromatography. As the binding sites inside of porous agarose beads are not accessible for cells, we engineered the beads to only enable surface-based binding of target molecules. In addition, their size was optimized for a steady flow of cells through the void space in the matrix without compromising the binding capacity. Lastly, the agarose has to be placed between two frits that allow an unrestricted flow of the cells, but still retain the beads in the column. To reversibly capture and release target cells, low affinity Fab fragments fused to a Twin-Strep-tag® (Fab-Streps) can be added to the TACS agarose filled columns **(Fig. 1).**

IBA provides Strep-Tactin® TACS agarose pre-packed in gravity flow columns, but also as agarose resin to make a multitude of applications possible. This

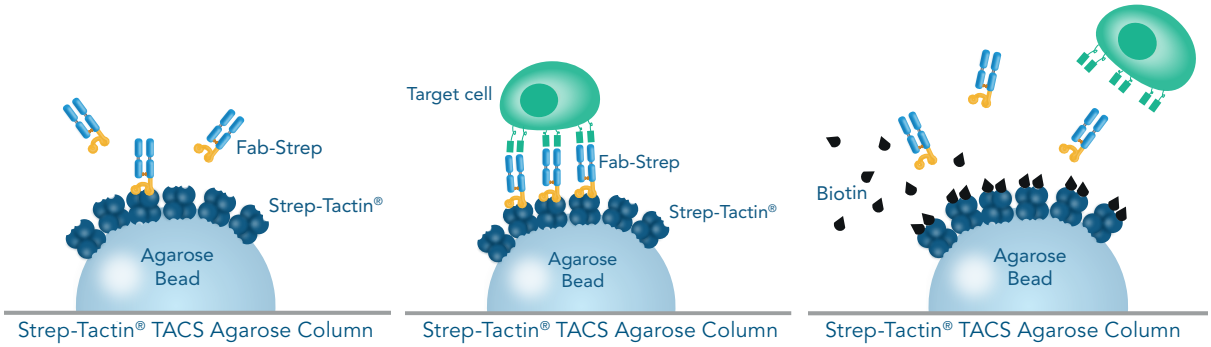

Fig. 1 Fab-based Traceless Affinity Cell Selection (Fab-TACS®): Strep-Tactin® coated agarose beads are loaded with low affinity Fab fragments fused to a Twin-Strep-tag® (Fab-Streps) against a cell type of choice. Subsequently, target cells are captured from a mixed population due to an increase in avidity caused by the multimerization of Fab-Streps on the agarose beads. Finally, cells are eluted from the beads with biotin. Biotin has a higher affinity to Strep-Tactin® than the Fab-Streps, causing their release from the beads. Due to the low affinity of the Fab-Streps to their target, they spontaneously dissociate from the cell surface, resulting in a label-free cell population.

protocol describes how to build columns for cell isolation to support different experimental approaches for Strep-Tactin® TACS Agarose. We also show exemplary data that were acquired by isolating cells with different volumes of the agarose bed, including a high-throughput approach using a pipetting robot (PhyNexus).

Methods

Building a column suitable for cell isolation – 5 ml bed volume

Two frits were incubated in 96% ethanol for 10 min to degas them **(Fig. 2A)**. 50 ml columns (Biocomma) were filled to the brim with cell isolation buffer (Buffer CI: PBS containing 0.5% BSA and 1 mM EDTA) **(Fig. 2B)** while avoiding air bubbles. The first frit was placed at the bottom of the column with a plunger **(Fig. 2C)**. 10 ml of Strep-Tactin® TACS Agarose (50% suspension, Cat. No.: 6-6350-010) was filled into the column **(Fig 2D).** After the agarose settled (about 15 min), the second degassed frit was put on top **(Fig. 2E)**.

Human CD3⁺ T cell isolation using a 5 ml Strep-Tactin® TACS Agarose Column

24 ml buffy coat was diluted with 16 ml Buffer CI. Columns were washed with 40 ml Buffer CI. 225 µg human CD3 Fab-Strep in 7 ml Buffer CI was loaded onto the column. After 2 min of incubation, columns were washed once with 10 ml Buffer CI. 20 ml diluted buffy coat was added to the column in two steps. After the sample passed the agarose bed completely, columns were washed three times with 50 ml Buffer CI. To elute bound cells, 5 ml Buffer CI with 1 mM biotin was added to the column. After 5 min of incubation, 20 ml Buffer CI with 1 mM biotin was added to the column once to collect all cells. Cell yield and purity were analyzed by flow cytometry.

Mouse CD4 Fab-Strep screening using the PhyNexus system

We used the PhyNexus MEA 2 system, which allows an automated isolation of up to 12 samples using flexible protocols with high-throughput. PhyTip® columns were packed with Strep-Tactin® TACS Agarose by putting an empty column into PBS up to half of its height. Afterwards, 160 µl of Strep-Tactin® TACS Agarose was added into the column. Finally, the top screen was placed on the resin using tweezers. The pre-packed columns were stored in PBS until usage.

Alternatively, custom (pre-packed) columns can be purchased from PhyNexus/Biotage with any desired resin.

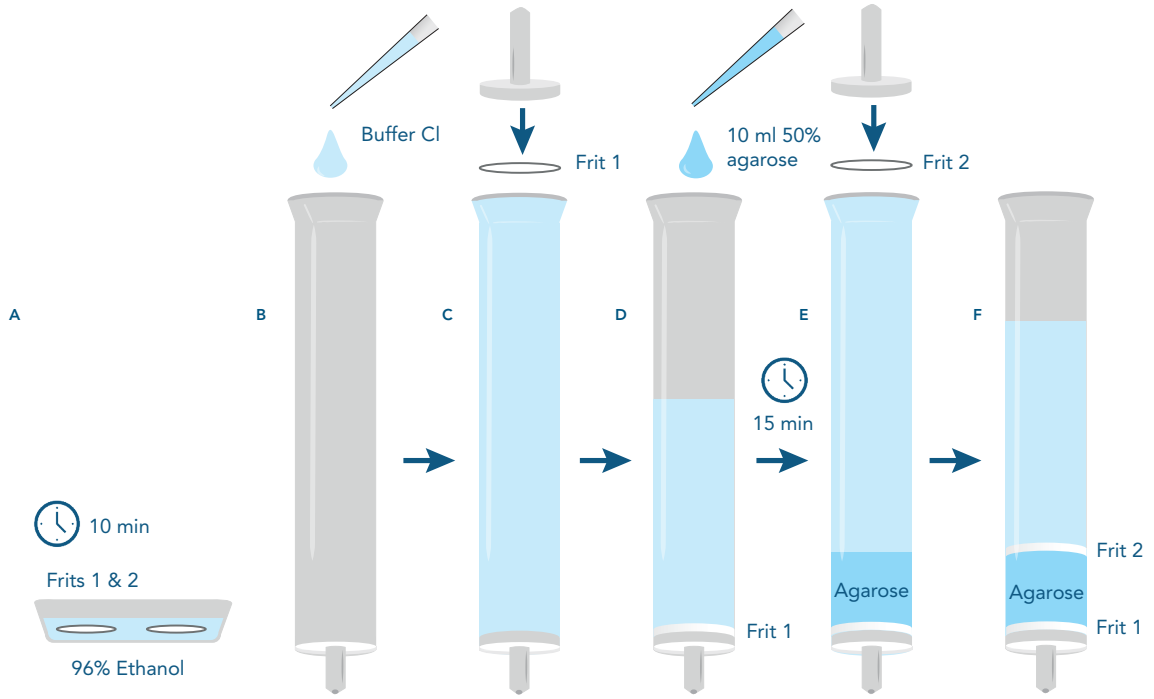

Fig. 2 Building a column with 5 ml Strep-Tactin® TACS Agarose. Two frits are incubated in ethanol to remove air bubbles (A). Columns are filled with Buffer CI (B) and the first frit is placed on the bottom (C). In a next step, Strep-Tactin® TACS Agarose is added (D). After the agarose settles down, columns are filled up with buffer CI and the second frit is placed on top (E). The assembled column (F) is applicable for cell isolation and cell depletion.

Mouse splenocytes (7 x 10^6 cells/ml in Buffer CI) were prepared. PhyNexus PhyTip® columns were loaded with three differently mutated mouse CD4 Fab-Streps (20 µg in 500 µl buffer CI per column). One negative control column without Fab-Streps was included. Bound cells were eluted with 2 ml of 1 mM biotin in Buffer CI. Isolation efficiency for each Fab-Strep was evaluated by flow cytometry.

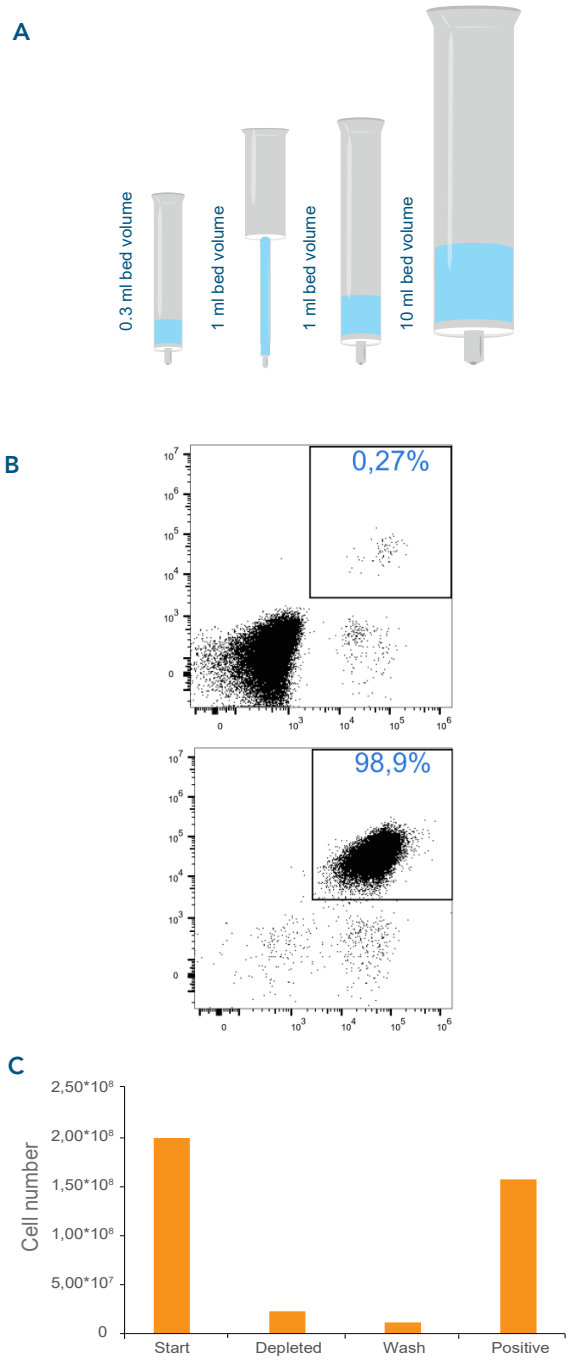

Fig. 3 Example of different shapes and sizes of gravity columns (A). Enrichment of CD3⁺ T Cells by using a 5 ml bed volume column with a purity of 98.9% (B) and a yield of 79% (C).

Results and discussion

CD3⁺ T cells are efficiently isolated using 5 ml bed volume columns

Strep-Tactin® TACS Agarose filled columns can be used to isolate specific cell populations from different samples. Depending on the sample size and amount of target cells, individual up- or downscaling of the amount of agarose might be necessary for efficient cell purification. Columns of different shapes and sizes can be packed **(Fig. 3A)** for various experimental approaches. The general packing procedure is described in **Figure 2.** We prepared a column with 5 ml bed volume and tested CD3⁺ T cell isolation from buffy coat using Fab-Streps. The purity was increased from 0.27% in the starting sample to 98.9% in the positive isolation fraction **(Fig 3B)**, while 79% of all T cells in the sample could be isolated **(Fig. 3C)**.

Strep-Tactin® TACS Agarose is applicable for automated systems

For some applications, only small amounts of isolated cells are needed. The automated PhyNexus system **(Fig. 4A)** works with small columns and is suitable for establishing screening assays, for example, to evaluate the performance of different Fab-Streps in cell isolation. For this approach, a bed volume of 160 µl is sufficient to isolate enough cells for flow

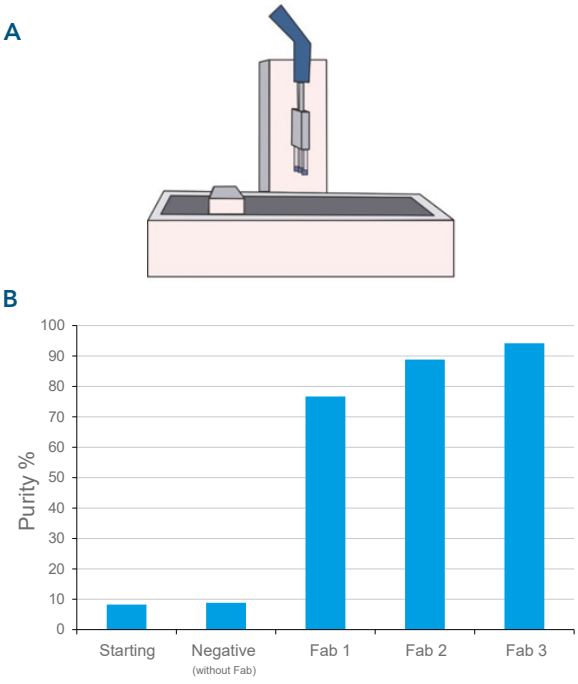

Fig. 4 The automated PhyNexus System (A) can be used to determine the best Fab-Strep in terms of target cell purity out of a various newly produced Fab-Streps. Three different CD4 Fab-Streps were used to isolate CD4⁺ T cells form murine splenocytes (B). Purity was determined by flow cytometry.

cytometry analysis. We tested this by isolating $CD4^+$ T cell from murine splenocytes using three different CD4 Fab-Streps. The purity of isolated cells ranged from 76.7% (Fab 1) to 94.2% (Fab 3, **Fig. 4B**), demonstrating that the PhyNexus system in combination with our Strep-Tactin® TACS Agarose is well suitable for evaluating the functionality of Strep-tagged proteins.

Conclusion

Our Strep-Tactin® TACS Agarose is applicable to individual cell isolation experiments due to the adaptability to different formats. Upscaling is easily achieved by packing columns with a custom amount of Strep-Tactin® TACS Agarose according to the expected amount of target cells. For downscaling or high-throughput screening, an automated system such as PhyNexus is a good choice. It offers an easy way to combine dual flow chromatography with the Fab-TACS® approach. This way, multiple cell samples can be tested in parallel using one type of Fab-Strep. Alternatively, multiple Fab-Streps can be characterized at the same time regarding their cell binding capacity.
Due to the flexible scaling possibilities and the ability to be functionalized with different proteins, Strep-Tactin® TACS Agarose is a versatile tool for various research projects.

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