

APPLICATION NOTE

Parallel purification of scFv antibody fragments using MAGicBeads

Introduction

The use of antibodies as tools in both basic research and clinical diagnostics is widespread. In addition, monoclonal antibodies for use as biopharmaceuticals are currently under heavy development globally. Recombinant DNA technology has enabled the use of *in vitro* selection methods, such as phage display technology, for efficient generation of antibody fragments, for example single chain fragment variable (scFv) antibodies, for specific antigens from large human antibody libraries. When such scFv fragment antibodies are constructed to contain a variable heavy domain of the IGHV3-gene, immobilized protein A can be used to purify these recombinantly expressed proteins in an efficient protocol giving highly pure protein. Herein, two different parallel purification setups, using MAGicBeads ProA (an early, non-alkali-tolerant predecessor of the current Protein A+ ligand), are tested on four 500 mL scFv protein bacterial preparations.

Purification of scFv from the periplasm using robotic system

The expressed scFv protein contains the ompA signal peptide, targeting the scFv protein to the periplasmic space. Bacteria were grown in 500 mL 2xYT medium, treated with IPTG to induce protein expression, and incubated for 20 h at 30°C, whereafter cells and media were separated by centrifugation. The bacterial pellets were lysed with 8 mL B-PER (Thermo Scientific) each and DNA nuclease and protease inhibitor added. Cell debris were pelleted by centrifugation and the supernatant added to a 15 mL tube containing 200 µL washed and settled MAGicBeads ProA particles, with four samples in parallel. Following 2 h incubation at room temperature (purification is briefly summarized in Table 1), the beads were captured using the MAGicAccio PILOT 50 separator, collected in 2 mL PBS, and transferred to a KingFisher™ 24 deep well plate (Thermo Fisher). Washing and elution were performed in a KingFisher Flex robot equipped with a 24 rod magnetic head, where an automated magnetic particle processing is used to move the magnetic MAGicBeads agarose particles from plate to plate. Adsorbed scFv protein was eluted twice in 2 mL low pH buffer, neutralized with 0.4 mL 2 M Tris-HCl, pH 9.0, and concentrated and changed into Dulbecco's PBS using Amicon Ultra-4 centrifuge filter units (Millipore).

Table 1. *Condition for purification from cell lysate*

Magnetic beads	MAGicBeads ProA (non-alkali-tolerant predecessor of Protein A+)
Sample	Total bacterial lysate
Bead volume	0.2 mL (2 mL 10% bead solution)
Sample volume	~ 10 mL
Magnetic separators	MAGicAccio PILOT 50 and KingFisher Flex robot (Thermo Fisher)
Binding conditions	B-PER buffer, 2 h, RT
Elution conditions	2x 2 mL acidic elution buffer (pH 3.0)
Neutralization	0.4 mL 2 M Tris-HCl, pH 9.0

Purification of scFv from bacterial culture media

After synthesis of the scFv protein by the bacteria, a portion of the protein escapes the periplasmic space and is found in the growth media. Adsorptions of scFv were therefore also tested directly from the cell culture media, as

summarized in Table 2. 200 µL washed and settled MAGicBeads ProA were mixed with approximately 500 mL scFv containing bacterial culture media for 20 h at 7°C, with four samples in parallel. Beads were first separated in a MAGicAccio PILOT 500 separator, resuspended in 40 mL PBS, and transferred to a 50 mL tube.
Beads were again separated, resuspended in a small volume PBS and transferred to a 2 mL microcentrifuge tube, where final washing took place. Adsorbed proteins were eluted, neutralized, and concentrated in buffers as described above.

The yield and purity of captured scFv fragments, from both purification setups, are given and visualized in Figures 1 and 2, respectively. Functional characterization of one individual clone by ELISA, is shown in Figure 3.

Table 2. *Condition for purification from cell media*

Magnetic beads	MAGicBeads ProA (non-alkali-tolerant predecessor of Protein A+)
Sample	Bacterial cell culture media
Bead volume	0.2 mL (2 mL 10% bead solution)
Sample volume	500 mL
Magnetic separators	MAGicAccio PILOT 50, MAGicAccio PILOT 500 and MAGicAccio LAB rack
Binding conditions	2xYT, 20 h, 7 °C
Elution conditions	2x 2 mL acidic elution buffer (pH 3.0)
Neutralization	0.4 mL 2 M Tris-HCl, pH 9.0

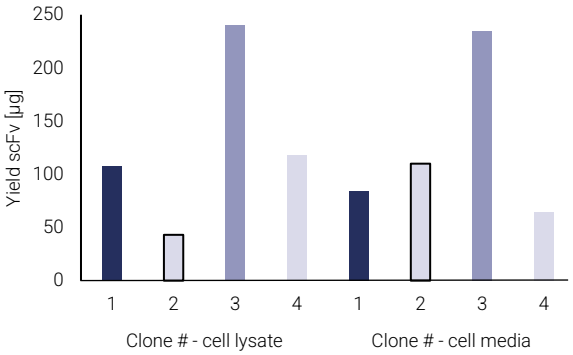

Figure 1. *Yield of scFv from either 10 mL clarified bacterial cell lysate using a KingFisher Flex robot or directly from 500 mlLcell culture media using the MAGicAccio PILOT 50 and 500 magnetic separators, and a magnetic microtube rack, MAGicAccio LAB rack.*

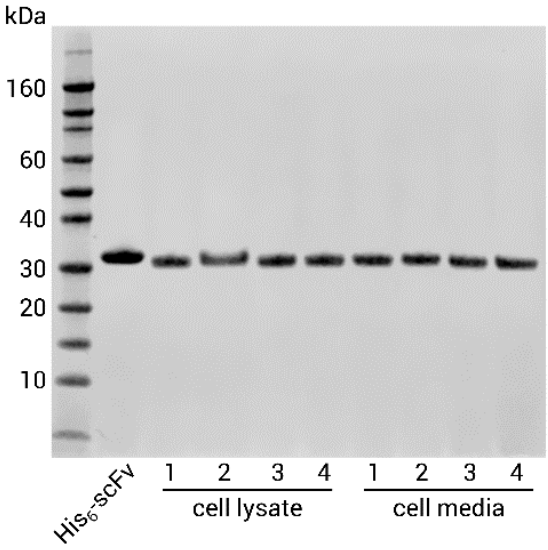

Figure 2. *Visual representation of purity for the four, different purified scFv proteins, using SDS-PAGE. Separated proteins were stained with SimplyBlue SafeStain (Thermo Fisher), and image capture performed with an Odyssey Fc (LI-COR Biosciences, UK). His6-scFv (3 µg) is an scFv-construct purified using IMAC, serving as a comparison control, and clones 1 to 4 (2 µg each), have been divided according to their prep methods, either from cell lysate or cell culture media, respectively. Size marker on the left.*

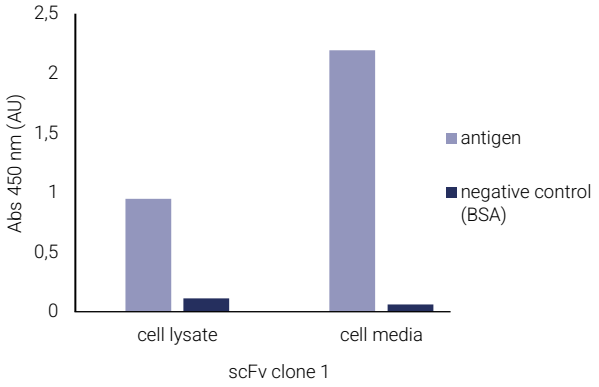

Figure 3*. Functional assessment of purified scFv clone 1 using ELISA. Wells were coated with 1 µg/mL of cognate antigen or BSA and incubated with 175 ng scFv. Functional scFv were detected with a secondary HRP-labelled anti-tag antibody, with absorbance at 450 nm as read out. Levels are presented as arbitrary units (AU).*

Discussion and Conclusion

Periplasmic targeted protein expression in bacteria, can have positive effects on folding and disulphide formation. Partial lysis of the bacteria can be used as one alternative to obtain the secreted protein, but, herein, complete lysis was performed with B-PER reagent, as a fast and efficient way to reach the expressed recombinant protein. Starting from four 500 mL induced bacterial cultures, purification of periplasmic targeted scFv fragments using MAGicBeads ProA magnetic agarose beads, proved successful from complete cell lysates. Separation of eluted material on SDS-PAGE, revealed very pure scFv proteins without any noticeable proteolytic degradation.

Parallel captures of the scFv that had leaked into the cell media, in the four 500 mL bacterial cell culture supernatants, were also straightforward, from adsorption to final elution. The purity of the obtained scFv antibodies were high, as indicated by SDS-PAGE. As exemplified with the ELISA test for scFv clone 1, the protein regained activity after being exposed to low pH during elution, with subsequent neutralization and buffer change.

Parallel purifications, using either setup, were straightforward, where the MAGicAccio PILOT 50 or 500 separators were used to initially capture the beads from 10 and 500 mL samples. Resuspension of the beads in small buffer volumes, made further automatic processing in the KingFisher Flex robot possible. The highly pure scFv antibodies are subsequently ready for various downstream functional assays.

*This study was conducted in collaboration between MAGic BioProcessing and the Drug Discovery & Development Platform at Science for Life Laboratory, together with the School of Biotechnology at KTH Royal Institute of Technology, Stockholm, Sweden. The academic collaborators were MSc Johannes Küpper and Dr. Helena Persson

MAGicBeads magnetic agarose beads enable robotic affinity purification of antibodies, antibody fragments, and fusion proteins using immobilized affinity ligands, such as Protein A or Protein G.

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