MAGic™ BioProcessing

APPLICATION NOTE

High-throughput purification of antibodies for candidate screening and clone selection

Introduction

The biologics market remains highly attractive to both small biotech companies and big pharma corporations where the products are made using recombinant technologies. Stresses are constantly increasing on the R&D to reduce the costs and timelines for taking new candidates from research phase into early clinical evaluation studies. Selection and isolation of stable high producer cell lines was for long addressed as a critical bottleneck, and the criteria for clone-selection have recently been expanded to also include product quality and functionality, driven by the increasing interest of biosimilars and biobetters. This has pave-the-way for multiparametric approaches that uses high-throughput, automated, microbioreactors in the Cell Line Development (CLD) stage.

The Ambr® 15 Cell Culture (Sartorius AG), implemented in CLD laboratories worldwide, is an automated bioreactor system for 24 parallel cultivations at the 10–15 mL scale. The advanced microbioreactor system enables high throughput screening to select the best clones, media, and feeds under small-scale bioreactor conditions, thus reducing the costs and timelines for early-stage process development.

Herein we demonstrate a complementary high-throughput platform to the Ambr15 Cell Culture system for product purification. This platform enables the further assessment of biological product quality and functionality. The described approach matches the number of bioreactors, culture volumes, cell titers and product concentrations that are obtained in the advanced microbioreactor system.

Experimental setup

The platform setup consists of magnetic beads together with a robotic magnetic extraction system to transfer the magnetic beads between sample, wash buffers and elution. The MAGic™Beads Protein A is super-paramagnetic porous agarose beads coupled with an alkali stable protein A ligand and is designed to simplify the purification process of antibodies by allowing capture directly from cell culture, eliminating the need of a cell clarification step.

We decided to use the magnetic-bead extraction instrument Maelstrom Switch 8 from TANBead as it matches the culture volumes (10-12 ml) as well as the product concentrations obtained with the Ambr15 system. The Switch 8 unit is designed to process 4 samples per run but is available in a 24 samples/run format (Maelstrom 2410) thus capable to purify all the 24 cell cultures obtained with the Ambr15 system in one go.

The automated extraction system captures and transfer the magnetic beads from well to well in a single 24 well format, as illustrated and described in Figure 1. Thus capture, washing and elution of the antibodies are performed on a single plate. The maximum capability of the instrument to extract and transfer the MAGicBeads without residual loss was determined to be 0.26 mL of sedimented beads per magnetic rod, corresponding to approximately 8 mg of IgG.

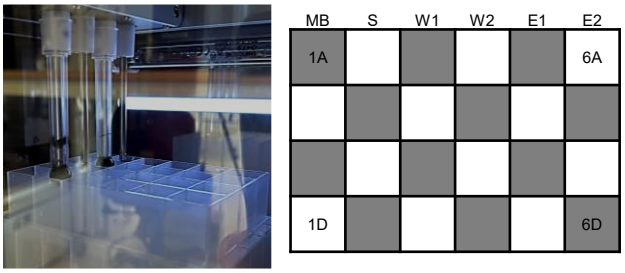

Figure 1. *Maelstrom Switch 8 magnetic bead extraction instrument. A) 4 magnetic rods are extracting and transferring magnetic beads between the wells performing capture, washing and elution. B) Orientation of magnetic beads (MB), sample (S), wash buffer (W1-W2), and elution buffer (E1-E2) on the 24 deep well plate.*

We decided to investigate the performance of the purification setup with 3 different product concentrations ranging from 0.03 to 1.30 mg IgG per mL of cell culture, thus including conditions from low, medium high producer cell lines. The POD clarified CHO-cell cultures were obtained from external sources.

The amount of affinity beads added to each IgG containing CHO-cell culture was determined as described in the user manual for MAGicBeads Protein A as the capacity of the magnetic bead resin is strongly dependent on the monoclonal antibody (mAb) concentration as shown in Figure 2.

The employed conditions as well as the time for the process to perform the purifications with the Switch 8 magnetic bead extraction instrument are more described in Table 1. We performed the purifications from the 3 different IgG containing CHO-cell cultures in duplicate. The final IgG concentrations was calculated by measuring the A_{280} value in the eluate fractions (deep-well columns 5-6).

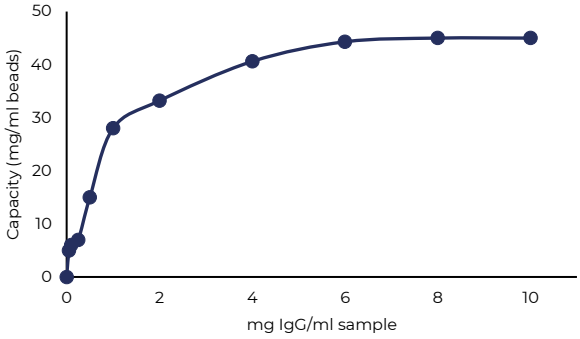

Figure 2. *Binding capacity of MAGicBeads Protein A as a function of IgG concentration in the sample.*

Table 1. *Purification conditions for the 3 different IgG containing cell cultures. bc = binding capacity*

Step Well column	0.027 mg IgG/mL	0.40 mg IgG/mL	1.30 mg IgG/mL
Magnetic bead collection W1	50 µL MAGicBeads Protein A bc@0.027mg/mL=5mg/mL beads 20 sec mixing @ 500rpm 20 sec collection with probe	0.26 mL MAGicBeads Protein A bc@0.40mg/mL=15mg/mL beads 20 sec mixing @ 500rpm 20 sec collection with probe	0.26 mL MAGicBeads Protein A bc@1.30mg/mL=28mg/mL beads 20 sec mixing @ 500rpm 20 sec collection with probe
Sample incubation W2	10 mL POD-filtered cell culture 0.27 mg IgG 1 hour mixing @ 500rpm 20 sec collection with probe	10 mL POD-filtered cell culture 4.0 mg IgG 1 hour mixing @ 500rpm 20 sec collection with probe	5.4 mL POD-filtered cell culture 7.0 mg IgG 1 hour mixing @ 500rpm 20 sec collection with probe
Wash W3-4	2x 3mL PBS 2x 20 sec mixing @ 500rpm 2x 20 sec collection with probe	2x 3mL PBS 2x 20 sec mixing @ 500rpm 2x 20 sec collection with probe	2x 3mL PBS 2x 20 sec mixing @ 500rpm 2x 20 sec collection with probe
Elution W5-6	2x 0.5mL 60mM citrate, pH 3.0 2x 5 min mixing @ 500rpm 2x 20 sec collection with probe	2x 1mL 60mM citrate, pH 3.0 2x 10 min mixing @ 500rpm 2x 20 sec collection with probe	2x mL 60mM citrate, pH 3.0 2x 10 min mixing @ 500rpm 2x 20 sec collection with probe
Total time	**1 h 13 min**	**1 h 23 min**	**1 h 23 min**

Table 2. *Purification results from the 3 different IgG containing cell cultures obtained with the magnetic bead extraction system. First row in each category displays results in 1st elution fractions. Second row, 2nd elution fractions. Third row, elution fractions pooled together.*

	0.027 mg IgG/mL	0.40 mg IgG/mL	1.30 mg IgG/mL
Recovered IgG	0.224 mg (1st elution) 0.044 mg (2nd elution) **0.267 mg (Total)**	3.14 mg 0.81 mg **3.95 mg**	5.58 mg 1.60 mg **7.18 mg**
IgG concentration in eluate	0.447 mg/mL 0.087 mg/mL **0.267 mg/mL**	3.14 mg/mL 0.81 mg/mL **1.97 mg/mL**	5.58 mg/mL 1.60 mg/mL **3.59 mg/mL**
Concentration factor	16.58 3.23 **9.9**	7.85 2.02 **5.06**	4.30 1.23 **2.76**

Results and discussion

The results for the purifications are shown in Table 2, and Figure 3 displays the yields as a function of IgG concentration in the CHO-cell cultures. High yields of 80% in the 1st elution fraction and nearly 100% in the pooled fractions were obtained for all the 3 investigated mAb concentrations spanning from low to high expression levels. The somewhat higher yields found for the low expressing CHO-cell culture can be explained with the higher elution volume compared to the amount of affinity beads used, e.g. 10x compared to 4x bead volume for the higher expression levels. The elution volumes for the latter are comparable to what you can expect for a column chromatography approach where you usually obtain the purified mAb in 3-4 column volumes. Ability to elute in somewhat small volumes enables the possibility for a large concentration effect where we obtained the low-expressed purified IgG 17x more concentrated compared to the input IgG titer (1st elution, 83% yield). For the high expressed IgG CHO-cell culture the concentration effect was 4.3 with a concentration of 5.58 mg IgG/mL eluate (1st elution, 79% yield). High concentrations for the purified molecule can be crucial for the further assessment of biological product quality and functionality. This study clearly shows that the demonstrated purification platform can be applied to both low and high expressed CHO-cell cultures obtaining high purification yields and high concentration effects. In approximately 1.5 hours, all 24 samples from an Ambr15 cell-culture system can be purified in one go.

Conclusion

- MAGicBeads Protein A in combination with Maelstrom magnetic bead extraction instrument provides a flexible mAb purification platform for 10-12 mL cell cultures
- Matching IgG-expressing cell cultures produced with the Ambr15 cell-culture system
  - Purify all 24 samples in one go
  - Process time 1.5 hours
- 80% purification yield in the first elution fraction
  - 99% yield in total
- Large concentration effects
  - 17x concentration for low IgG-expressing cell cultures

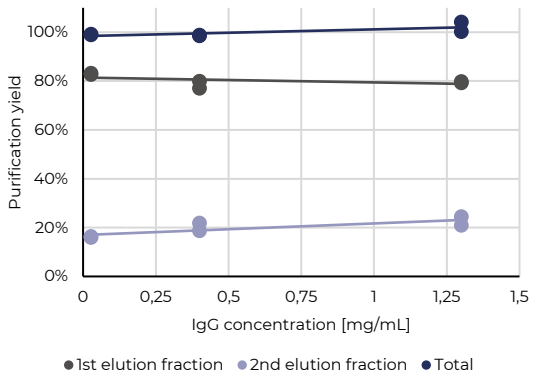

Figure 3. *Purification yields from the 3 different CHO cell cultures expressing IgG at different expression levels at 0.027, 0.40 and 1.30 mg/mL.*

Ordering information

Visit www.magicbioprocessing.com for information regarding all MAGicBeads resins and MAGicAccio units.

Orders via web shop or your local distributor.

Contact us via info@magicbioprocessing.com

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